

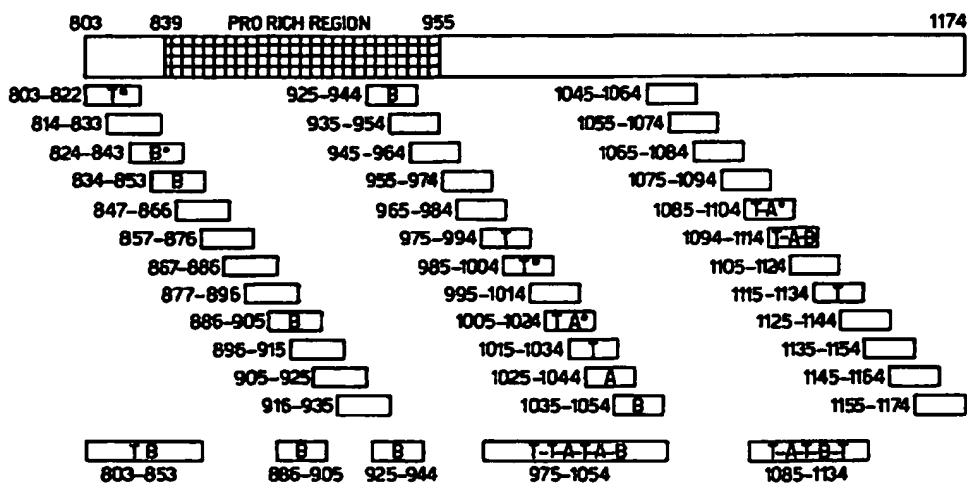


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## (57) Abstract

A nucleic acid sequence which codes upon expression in a prokaryotic or eukaryotic host cell for a polypeptide product having one or more properties selected from (i) the ability to adhere to a mammalian tooth in a competitive manner with naturally occurring *Streptococcus mutans* antigen I/II, thus preventing or diminishing the adhesion of *S. mutans* to the tooth; (ii) the ability to stimulate a T-cell response; and (iii) the ability to stimulate a B-cell response, said nucleic acid sequence being selected from: (a) the sequences shown in SEQ. ID. No. 12 to 22 or the complementary strands thereof; (b) nucleic acid sequences having a length of not more than 1000 base pairs which hybridise to the sequences defined in (a) over at least 70 % of their length; (c) nucleic acid sequences having a length of not more than 1000 base pairs which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid sequences defined in (a) or (b) over at least 70 % of their length and which sequences code for polypeptides having the same amino acid sequence.

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**POLYPEPTIDE FRAGMENTS CAPABLE OF COMPETITION WITH STREPTOCOCCUS MUTANS ANTIGEN I/II**

This invention relates to polypeptide fragments of the Streptococcus mutans I/II antigen that are useful in  
5 treating and preventing dental caries.

Streptococcus mutans is the main etiological agent of dental caries, a disease which affects mammals including humans.

The S. mutans I/II antigen (SA I/II) is a cell  
10 surface protein with an  $M_r$  of about 185kDa. It is believed to comprise several antigenic epitopes and to be at least partly responsible for S. mutans adhesion to teeth.

SA I/II is described in British Patent No. 2,060,647, as are number antibodies to it. A putative 3.5 to 4.5 kDa  
15 fragment of SA I/II, "antigen X", has also been described in European Patent No. 0 116 472.

However, it has now become clear that "antigen X" is not a fragment of SA I/II at all. Rather, it is a separate protein that merely co-purifies with SA I/II. It is  
20 believed to be encoded by a separate gene.

Two large fragments of SA I/II, an N-terminal fragment (residues 39 to 481) and a 40kDa central fragment (residues 816 to 1213) are recognised by human serum antibodies. Within the central fragment, 80% of the sera  
25 tested recognise elements within a proline-rich region (residues 839-955) that comprises three tandem repeats. This suggests that this region includes one or more B-cell epitopes. The central fragment (residues 816-1213) is also believed to comprise one or more adhesion sites that  
30 mediate S. mutans' attachment to the tooth.

The aim of the above-mentioned work has been the development of vaccines for immunisation against dental caries. However, precise identification of the antigenic epitopes within SA I/II is a prerequisite for designing

synthetic vaccines based on it. Similarly, precise identification of adhesion sites is essential for the design of drugs against dental caries that rely on inhibiting S. mutans' adhesion to the tooth.

5           No antigenic epitopes (T-cell or B-cell epitopes) or adhesion sites within SA I/II have been characterised, nor has the precise location of any such regions been suggested. Also, there has been no indication of the location of S. mutans' T-cell epitopes as the above-  
10 mentioned work has concentrated on S. mutans' ability to adhere to teeth and to generate a B-cell response.

          The inventors have identified a number of T-cell epitopes, B-cell epitopes and adhesion sites within residues 803 to 1114 of SA I/II. Some of the T-cell and B-  
15 cell epitopes overlap or are contiguous with each other and/or with one or more of the adhesion sites.

          The presence of a number of antigenic epitopes of both types and a number of adhesion sites within the same region of SA I/II could not have been predicted and the  
20 finding that some of the adhesion sites and epitopes overlap or are contiguous with each other is particularly surprising.

          These findings make it possible to design effective synthetic vaccines against dental caries as well as drugs  
25 that engender resistance against the disease or alleviate pre-existing cases of it by preventing S. mutans' adhesion to the tooth. Further, the surprising finding that some of the T-antigenic epitopes and the adhesion site are contiguous or overlapping makes it possible to design  
30 bifunctional drugs that effect immunisation against dental caries as well as preventing adhesion of S. mutans to the tooth.

          Accordingly, the present invention provides a nucleic acid sequence which codes upon expression in a prokaryotic

or eukaryotic host cell for a polypeptide product having one or more properties selected from (i) the ability to adhere to a mammalian tooth in a competitive manner with naturally occurring Streptococcus mutans antigen I/II, thus preventing or diminishing the adhesion of S. mutans to the tooth; (ii) the ability to stimulate a T-cell response; and (iii) the ability to stimulate a B-cell response, said nucleic acid sequence being selected from:

- 10 (a) the sequences shown in SEQ. ID. Nos. 12 to 22 or the complementary strands thereof;
- (b) nucleic acid sequences having a length of not more than 1000 base pairs which hybridise to the sequences defined in (a) over at least 70% of their length;
- 15 (c) nucleic acid sequences having a length of not more than 1000 base pairs which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid sequences defined in (a) or (b) over at least 70% of their length and which sequences code
- 20 for polypeptides having the same amino acid sequence code, would hybridise to the nucleic acid sequences defined in (a) or (b) over at least 70% of their length and which sequences code for polypeptides having the same amino acid sequence.

25

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Depiction of the panel of overlapping 20mers used to map T-cell, B-cell and adhesion epitopes within SA I/II.

30

Figure 2. Proliferative responses to overlapping synthetic peptides (20<sup>mers</sup>) of SA I/II.

- a) Mean S.I. ( $\pm$ sem) of PBMC from 30 subjects. Mean cpm with medium only was 538 $\pm$ 112.

- b) Frequency of positive responses ( $S.I. \geq 3.0$ ,  $cpm > 500$ ).

**Figure 3.** MHC class II dependency of proliferative responses to SA I/II.

5

**Figure 4.** Serum recognition of SA I/II and recombinant polypeptide fragments. Western blots from 3 subjects are shown (panels a-c) together with rabbit anti-SA I/II antiserum (panel d). Lanes, 1, SA I/II; lane 5,

10 recombinant 984-1161.

**Figure 5.** Human serum recognition of synthetic peptides of SA I/II.

- 15 a) Titres were determined by ELISA in 22 subjects to selected peptides of SA I/II and an irrelevant control peptide from SIVp27(SIV). The frequencies of sera binding the peptides with a titre  $> \text{mean} + 2S.D.$  the control peptide are also indicated.

20

**Figure 6.** Inhibition of adhesion of S. mutans.

- a) SA I/II and recombinant fragment 984-1161.  
b) Synthetic peptides.

- 25 **Figure 7.** Proliferative responses of murine splenocytes following immunization with recombinant 975-1044 (SEQ. ID. No. 8)

- Figure 8.** Competitive inhibition of SA I/II binding by  
30 various polypeptides.

**Figure 9.** Dependence of competitive inhibition of SA I/II binding on concentration of two peptides.

Figure 10. Effects of substitution of certain residues on competitive inhibition.

Figure 11. Comparison of various recombinant polypeptides  
5 with respect to binding.

The polypeptides of the invention have one or more of the following properties. Firstly, they may have the ability to adhere to a mammalian tooth in a competitive  
10 manner with naturally occurring Streptococcus mutans antigen I/II, thus preventing or diminishing the adhesion of S. mutans to the tooth. Some of the peptides of the invention have been shown to inhibit adhesion of S. mutans to a tooth surface model (whole human saliva adsorbed to  
15 the wells of polystyrene microtitre plates or hydroxyapatite beads). Thus, these peptides comprise one or more adhesion sites and will adhere to a mammalian tooth in a competitive manner with naturally occurring SA I/II. Therefore, peptides according to the invention that  
20 comprise the adhesion site prevent or diminish the adhesion of S. mutans to the tooth. Peptides of the invention that comprise one or more adhesion epitopes include SEQ. ID. Nos. 1 to 6 and 8 to 10.

Secondly, peptides according to the invention may  
25 have the ability to stimulate a T-cell response. The inventors have shown that residues 803 to 854 and 925 to 1114 of SA I/II comprise a number of T-cell epitopes that are at least partially responsible for the T-cell response stimulated by the intact protein. Therefore, peptides  
30 according to the invention that comprise one or more of these the T-cell epitopes stimulate a T-cell response against S. mutans infection. Peptides of the invention that stimulate a T-cell response include those shown in SEQ ID Nos. 1 to 11.

Thirdly, the peptides of the invention may stimulate a B-cell response. The inventors have shown that residues 803 to 854 and 925 to 1114 of SA I/II comprise a number of B-cell epitopes and polypeptides according to the invention  
5 that comprise one or more B-cell epitopes stimulate a B-cell response against S. mutans infection. Peptides of the invention that comprise one or more B-cell epitopes include those shown in SEQ. ID. Nos. 1, 3 to 7 and 10.

The nucleic acid sequences of the present invention  
10 are preferably DNA, though they may be RNA. It will be obvious to those of skill in the art that, in RNA sequences according to the invention, the T residues shown in SEQ. ID. Nos. 12 to 22 will be replaced by U. Nucleic acid sequences of the invention will typically be in isolated or  
15 substantially isolated form. For example up to 80, up to 90, up to 95 or up to 100% of the nucleic acid material in a preparation of a nucleic acid of the invention will typically be nucleic acid according to the invention.

Some preferred nucleic acid sequences of the  
20 invention are those shown in SEQ. ID. Nos. 12 to 22. However, the nucleic acid sequences of the present invention are not limited to these sequences. Rather, the sequences of the invention include sequences that are closely related to these sequences and that encode a  
25 polypeptide having at least one of the biological properties of naturally occurring SA I/II. These sequences may be prepared by altering those of SEQ ID Nos. 12 to 22 by any conventional method, or isolated from any organism or made synthetically. Such alterations, isolations or  
30 syntheses may be performed by any conventional method, for example by the methods of Sambrook et al (Molecular Cloning: A Laboratory Manual; 1989)

For example, the sequences of the invention include sequences that are capable of selective hybridisation to



those of SEQ. ID. Nos. 12 to 22 or the complementary strands thereof and that encode a polypeptide having one or more of the properties defined above. Such sequences capable of selectively hybridizing to the DNA of SEQ. ID.

- 5 Nos. 12 to 22 will generally be at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the DNA of SEQ. ID. Nos. 12 to 22 over a region of at least 10, preferably at least 20, 30, 40, 50 or more contiguous nucleotides.

- 10 Such sequences that hybridise to those shown in SEQ. ID. Nos. 12 to 22 will typically be of similar size to them, though they may be longer or shorter. However, if they are longer, they may not simply encode large fragments of native SA I/II amino acid sequence. Thus, sequences
- 15 that hybridise to those of SEQ. ID. Nos. 12 to 22 may be sequences of up to 1000 bases in length, for example up to 950 or up to 933 bases in length, 933 bases being the length of the DNA sequence encoding the largest specifically identified peptide of the invention (SEQ. ID.
- 20 No. 21). Also, sequences that hybridise to those of SEQ. ID. Nos. 12 to 22 must do so over at least 50% of their length, for example up to 60%, up to 70%, up to 80%, up to 90%, up to 95%, or up to 99% of their length.

- Such hybridisation may be carried out under any
- 25 suitable conditions known in the art (see Sambrook et al (1989): Molecular Cloning: A Laboratory Manual). For example, if high stringency is required, suitable conditions include 0.2 x SSC at 60°C. If lower stringency is required, suitable conditions include 2 x SSC at 60°C.

- 30 Also included within the scope of the invention are sequences that differ from those defined above because of the degeneracy of the genetic code and encode the same polypeptide having one or more of the properties defined above, namely the polypeptide of SEQ. ID. Nos. 1 to 11 or

a polypeptide related to one of these polypeptides in any of the ways defined below.

Thus, the nucleic acid sequences of the invention include sequences which, but for the degeneracy of the genetic code, would hybridise to those shown in SEQ. ID. Nos. 12 to 22 or the complementary strands thereof. However, such sequences may not simply encode large fragments of native SA I/II amino acid sequences. Thus, these sequences may be up to 1000 bases in length, for example up to 950 or 933 bases in length. Also, their sequence must be such that, but of the degeneracy of the genetic code, they would hybridise to a sequence as shown in SEQ. ID. Nos. 12 to 22 over at least 50% of their length, for example, up to 60%, up to 70%, up to 80%, up to 90%, up to 95% or up to 99% of their length.

Also, the nucleic acid sequences of the invention include the complementary strands of the sequences defined above, for example the complementary strands of the nucleic acid sequences shown in SEQ. ID. Nos. 12 to 22.

Nucleic acid sequences of the invention will preferably be at least 30 bases in length, for example up to 50, up to 100, up to 200, up to 300, up to 400, up to 500, up to 600, up to 800 or up to 1000 bases.

Nucleic acid sequences of the invention may be extended at either or both of the 5' and 3' ends. Such extensions may be of any length. For example, an extension may comprise up to 10, up to 20, up to 50, up to 100, up to 200 or up to 500 or more nucleic acids. A 5' extension may have any sequence apart from that which is immediately 5' to the sequence of the invention (or the native sequence from which it is derived) in native SA I/II. A 3' extension may have any sequence apart from that which is 3' to the sequence of SEQ. ID. No. 13 in native SA I/II. Thus, the nucleic acid sequences of the invention may be

extended at either or both of the 5' and 3' ends by any non-wild-type sequence.

The polypeptides of the invention are encoded by the DNA sequences described above. Thus, the polypeptides of the invention are not limited to the polypeptides of SEQ. ID. Nos. 1 to 11 although these sequences represent preferred polypeptides. Rather, the polypeptides of the invention also include polypeptides with sequences closely related to those of SEQ. ID. Nos. 1 to 11 that have one or more of the biological properties of SA I/II. These sequences may be prepared by altering those of SEQ ID Nos. 1 to 11 by any conventional method, or isolated from any organism or made synthetically. Such alterations, isolations or syntheses may be performed by any conventional method, for example by the methods of Sambrook et al (Molecular Cloning: A Laboratory Manual; 1989). In particular, polypeptides related to those of SEQ ID Nos. 1 to 11 may be prepared by modifying DNA sequences as shown in SEQ ID Nos. 12 to 22 expressing them recombinantly.

The polypeptides of the invention may be encoded by nucleic acid sequences that have less than 100% sequence identity with those of SEQ. ID. Nos. 12 to 22. Thus, polypeptides of the invention may include substitutions, deletions, or insertions, that distinguish them from SEQ. ID. Nos. 1 to 11 as long as these do not destroy the biological property or properties that the polypeptides have in common with SA I/II.

A substitution, deletion or insertion may suitably involve one or more amino acids, typically from one to five, one to ten or one to twenty amino acids, for example, a substitution, deletion or insertion of one, two, three, four, five, eight, ten, fifteen, or twenty amino acids. Typically, a polypeptide of the invention has at least 60%, at least 80%, at least 90%, or at least 95% sequence

identity to the sequence of any one of SEQ. ID. Nos. 1 to 11.

In general, the physicochemical nature of the sequence of SEQ. ID. Nos. 1 to 11 should be preserved in a polypeptide of the invention. Such sequences will generally be similar in charge, hydrophobicity and size to that of SEQ. ID. Nos. 1 to 11. Examples of substitutions that do not greatly affect the physicochemical nature of amino acid sequences are those in which an amino acid from one of the following groups is substituted by a different amino acid from the same group:

H, R and K  
I, L, V and M  
15 A, G, S and T  
D, E, Q and N.

However, it may be desirable to alter the physicochemical nature of the sequence of SEQ. ID. Nos. 1 to 11 in order to increase its therapeutic effectiveness. For example, many of the amino acids in the polypeptides of the invention are acidic. For example, residues 975 to 1044 (SEQ. ID. No. 8) as a whole are of an acidic nature. This acidity is believed to facilitate binding to a mammalian tooth. Thus, it may be desirable to increase the acidity of polypeptides of the invention by adding acidic residues or by substituting acidic residues for non-acidic ones. Acidic residues include aspartic acid and glutamic acid.

30 Where polypeptides of the invention are synthesised chemically, D-amino acids (which do not occur in nature) may be incorporated into the amino acid sequence at sites where they do not affect the polypeptides biological properties. This reduces the polypeptides' susceptibility

to proteolysis by the recipient's proteases.

The nucleic acid sequences encoding the polypeptides of the invention may be extended at one or both ends by any non-wild-type sequence.

5        Thus, the polypeptides of the invention may be extended at either or both of the C- and N- termini by an amino acid sequence of any length. For example, an extension may comprise up to 5, up to 10, up to 20, up to 50, or up to 100 or 200 or more amino acids. An N-terminal  
10 extension may have any sequence apart from that which is N-terminal to the sequence of SEQ. ID. No. the invention (or the native sequence from which it is derived) in native SA I/II. A C-terminal extension may have any sequence apart from that which is C-terminal to the sequence of the  
15 invention (or the native sequence from which it is derived) in native SA I/II. Thus, the polypeptides of the invention may be extended at either or both of the C- and N- termini by any non-wild-type sequence.

      The polypeptides of the invention may be attached to  
20 other polypeptides or proteins that enhance their antigenic properties. Thus, polypeptides of the invention may be attached to one or more other antigenic polypeptides. These additional antigenic polypeptides may be derived from S. mutans or from another organism. Possible additional  
25 antigenic polypeptides include heterologous T-cell epitopes derived from other S. mutans proteins or from species other than S. mutans. Heterologous B-cell epitopes may also be used. Such heterologous T-cell and or B-cell epitopes may be of any length and epitopes of up to 5, up to 10 or up to  
30 20 amino acids in length are particularly preferred. These additional antigenic polypeptides may be attached to the polypeptides of the invention chemically. Alternatively, one or more additional antigenic sequences may comprise an extension to a polypeptide of the invention.

A polypeptide of the invention may be subjected to one or more chemical modifications, such as glycosylation, sulphation, COOH-amidation or acylation. In particular, polypeptides that are acetylated at the N-terminus are preferred, as are polypeptides having C-terminal amide groups. Preferred polypeptides may have one or more of these modifications. For example, particularly preferred peptides may have a C-terminal amide group and N-terminal acetylation.

10 A polypeptide of the invention may form part of a larger polypeptide comprising multiple copies of the sequence of one or more of SEQ. ID. Nos. 1 to 11 or a sequences related to them in any of the ways defined herein.

15 Polypeptides of the invention typically comprise at least 15 amino acids, for example 15 to 20, 20 to 50, 50 to 100 or 100 to 200 or 200 to 300 amino acids. Preferred polypeptides include those shown in SEQ. ID. Nos. 1 to 11.

Polypeptides according to the invention may be purified or substantially purified. Such a polypeptide in substantially purified form will generally form part of a preparation in which more than 90%, for example up to 95%, up to 98% or up to 99% of the peptide material in the preparation is that of a polypeptide or polypeptides according to the invention.

25 The nucleic acid sequences and polypeptides of the invention were originally derived from S. mutans. However, nucleic acid sequences and/or polypeptides of the invention may also be obtained from other organisms, typically bacteria, especially other streptococci. They may be obtained either by conventional cloning techniques or by probing genomic or cDNA libraries with nucleic acid sequences according to the invention. This can be done by any conventional method, such as the methods of Sambrook et

al (Molecular Cloning: A Laboratory Manual; 1989).

A nucleic acid sequence according to the invention may be included within a vector, suitably a replicable vector, for instance a replicable expression vector.

5 A replicable expression vector comprises an origin of replication so that the vector can be replicated in a host cell such as a bacterial host cell. A suitable vector will also typically comprise the following elements, usually in a 5' to 3' arrangement: a promoter for directing  
10 expression of the nucleic acid sequence and optionally a regulator of the promoter, a translational start codon and a nucleic acid sequence according to the invention encoding a polypeptide having one or more of the biological properties of SA I/II. A non-replicable vector lacks a  
15 suitable origin at replication whilst a non-expression vector lacks an effective promoter.

The vector may also contain one or more selectable marker genes, for example an ampicillin resistance gene for the identification of bacterial transformants. One  
20 particular preferred marker gene is the kanamycin resistance gene. Optionally, the vector may also comprise an enhancer for the promoter. If it is desired to express the nucleic acid sequence of the invention in a eucaryotic cell, the vector may also comprise a polyadenylation signal  
25 operably linked 3' to the nucleic acid encoding the functional protein. The vector may also comprise a transcriptional terminator 3' to the sequence encoding the polypeptide of the invention.

The vector may also comprise one or more non-coding  
30 sequences 3' to the sequence encoding the polypeptide of the invention. These may be from S. mutans (the organism from which the sequences of the invention are derived) or the host organism which is to be transformed with the vector or from another organism.

In an expression vector, the nucleic acid sequence of the invention is operably linked to a promoter capable of expressing the sequence. "Operably linked" refers to a juxtaposition wherein the promoter and the nucleic acid sequence encoding the polypeptide of the invention are in a relationship permitting the coding sequence to be expressed under the control of the promoter. Thus, there may be elements such as 5' non-coding sequence between the promoter and coding sequence. These elements may be native either to S. mutans or to the organism from which the promoter sequence is derived or to neither organism. Such sequences can be included in the vector if they enhance or do not impair the correct control of the coding sequence by the promoter.

The vector may be of any type. The vector may be in linear or circular form. For example, the vector may be a plasmid vector. Those of skill in the art will be able to prepare suitable vectors comprising nucleic acid sequences encoding polypeptides of the invention starting with widely available vectors which will be modified by genetic engineering techniques such as those described by Sambrook et al (Molecular Cloning: A Laboratory Manual; 1989). Preferred starting vectors include plasmids that confer kanamycin resistance and direct expression of the polypeptide of the invention via a tac promoter.

In an expression vector, any promoter capable of directing expression of a sequence of the invention in a host cell may be operably linked to the nucleic acid sequence of the invention. Suitable promoters include the tac promoter.

Such vectors may be used to transfect or transform a host cell. Depending on the type of vector, they may be used as cloning vectors to amplify DNA sequences according to the invention or to express this DNA in a host cell.



A further embodiment of the invention provides host cells harbouring vectors of the invention, i.e. cells transformed or transfected with vectors for the replication and/or expression of nucleic acid sequences according to the invention, including the sequences shown in SEQ. ID. Nos. 12 to 22. The cells will be chosen to be compatible with the vector and may for example be bacterial cells. Transformed or transfected bacterial cells, for example E.coli cells, will be particularly useful for amplifying nucleic acid sequences of the invention as well as for expressing them as polypeptides.

The cells may be transformed or transfected by any suitable method, such as the methods described by Sambrook et al (Molecular cloning: A Laboratory Manual; 1989). For example, vectors comprising nucleic acid sequences according to the invention may be packaged into infectious viral particles, such as retroviral particles. The constructs may also be introduced, for example, by electroporation, calcium phosphate precipitation, biolistic methods or by contacting naked nucleic acid vectors with the cells in solution.

In the said nucleic acid vectors with which the host cells are transformed or transfected, the nucleic may be DNA or RNA, preferably DNA.

The vectors with which the host cells are transformed or transfected may be of any suitable type. The vectors may be able to effect integration of nucleic acid sequences of the invention into the host cell genome or they may remain free in the cytoplasm. For example, the vector used for transformation may be an expression vector as defined herein.

The present invention also provides a process of producing polypeptides according to the invention. Such a process will typically comprise transforming or

transfecting host cells with vectors comprising nucleic acid sequences according to the invention and expressing the nucleic acid sequence in these cells. In this case, the nucleic acid sequence will be operably linked to a  
5 promoter capable of directing its expression in the host cell. Desirably, such a promoter will be a "strong" promoter capable of achieving high levels of expression in the host cell. It may be desirable to overexpress the polypeptide according to the invention in the host cell.  
10 Suitable host cells for this purpose include yeast cells and bacterial cells, for example E. coli cells, a particularly preferred E. coli strain being E. coli K12 strain BL 21. However, other expression systems can also be used, for example baculovirus systems in which the  
15 vector is a baculovirus having in its genome nucleic acid encoding a polypeptide of the invention and expression occurs when the baculovirus is allowed to infect insect cells.

The thus produced polypeptide of the invention may be  
20 recovered by any suitable method known in the art. Optionally, the thus recovered polypeptide may be purified by any suitable method, for example a method according to Sambrook et al (Molecular Cloning: A Laboratory Manual).

The polypeptides of the invention may also be  
25 synthesised chemically using standard techniques of peptide synthesis. For shorter polypeptides, chemical synthesis may be preferable to recombinant expression. In particular, peptides of up to 20 or up to 40 amino acid residues in length may desirably be synthesised chemically.

30 The nucleic acid sequences of the invention may be used to prepare probes and primers. These will be useful, for example, in the isolation of genes having sequences similar to that of SEQ. ID. No. 24. Such probes and primers may be of any suitable length, desirably from 10 to

100, for example from 10 to 20, 20 to 50 or 50 to 100 bases in length.

The present invention also provides antibodies to the polypeptides of the invention. These antibodies may be  
5 monoclonal or polyclonal. For the purposes of this invention, the term "antibody", includes fragments of whole antibodies which retain their binding activity for a target antigen. Such fragments include Fv, F(ab') and F(ab')<sub>2</sub> fragments, as well as single chain antibodies.

10 The antibodies may be produced by any method known in the art, such as the methods of Sambrook et al (Molecular Cloning: A Laboratory Manual; 1989). For example, they may be prepared by conventional hybridoma techniques or, in the case of modified antibodies or fragments, by recombinant  
15 DNA technology, for example by the expression in a suitable host vector of a DNA construct encoding the modified antibody or fragment operably linked to a promoter. Suitable host cells include bacterial (for example E. coli), yeast, insect and mammalian cells. Polyclonal  
20 antibodies may also be prepared by conventional means which comprise inoculating a host animal, for example a rat or a rabbit, with a peptide of the invention and recovering immune serum.

The present invention also provides pharmaceutical  
25 compositions comprising polypeptides of the invention. Three types of pharmaceutical compositions are particularly preferred. Firstly, compositions comprising polypeptides of the invention that include T-cell and/or B-cell epitopes may be used as vaccines against dental caries. Secondly,  
30 compositions comprising polypeptides of the invention that comprise adhesion sites will prevent or diminish adhesion of S. mutans to the tooth and can be used in the treatment of pre-existing cases of dental caries. Thirdly, compositions comprising polypeptides of the invention that

include both one or more antigenic (T-cell or B-cell) epitopes and one or more adhesion epitopes can be used to effect vaccination against dental caries at the same time as caring pre-existing cases of the disease. A similar  
5 effect can be achieved by including in a composition one or more peptides comprising one or more antigenic epitopes and one or more peptides comprising one or more adhesion sites.

A range of mammalian species can be vaccinated against dental caries using the polypeptides of the  
10 invention. Vaccination of humans is particularly desirable.

The compositions of the invention may be administered to mammals including humans by any route appropriate. Suitable routes include topical application in the mouth,  
15 oral delivery by means of tablets or capsule and parenteral delivery, including subcutaneous, intramuscular, intravenous and intradermal delivery. Preferred routes of administration are topical application in the mouth and injection, typically subcutaneous or intramuscular  
20 injection, with a view to effecting systemic immunisation.

As previously indicated, polypeptides according to the invention may also be mixed with other antigens of different immunogenicity.

The compositions of the invention may be administered  
25 to the subject alone or in a liposome or associated with other delivery molecules. The effective dosage depends on many factors, such as whether a delivery molecule is used, the route of delivery and the size of the mammal being vaccinated. Typical doses are from 0.1 to 100 mg of the  
30 polypeptide of the invention per dose, for example 0.1 to 1mg, and 1 to 5mg, 5 to 10mg and 10 to 100mg per dose. Doses of from 1 to 5 mg are preferred.

Dosage schedules will vary according to, for example, the route of administration, the species of the recipient

and the condition of the recipient. However, single doses and multiple doses spread over periods of days, weeks or months are envisaged. A regime for administering a vaccine composition of the invention to young human patients will  
5 conveniently be :6 months, 2 years, 5 years and 10 years, with the initial dose being accompanied by adjuvant and the subsequent doses being about  $\frac{1}{4}$  to  $\frac{1}{2}$  the level of polypeptide in the initial dose. The frequency of administration can, however, be determined by monitoring  
10 the antibody levels in the patient.

Where the peptides of the invention are to be applied topically in the mouth, one preferred dosage regime is to apply one or more polypeptides of the invention on two or more occasions, for example 2 to 10 occasions over a period  
15 of a few weeks, for example one to six weeks. A particularly preferred regime of this type involves six applications of a polypeptide of the invention over a period of three weeks.

Typical doses for each topical application are in the  
20 range of 0.1 to 100mg for example 0.1 to 1mg, 1 to 10mg and 10 to 100mg. Doses of from 1 to 5mg for each application are preferred.

While it is possible for polypeptides of the invention to be administered alone it is preferable to  
25 present them as pharmaceutical formulations. The formulations of the present invention comprise at least one active ingredient, a polypeptide of the invention, together with one or more acceptable carriers thereof and optionally other therapeutic ingredients. The carrier or carriers  
30 must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipients thereof, for example, liposomes.

Formulations suitable for parenteral administration

include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostatis, bactericidal antibiotics and solutes which render the formulation isotonic with the blood of the intended  
5 recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs.

10 In particular, the polypeptides of the invention may be coupled to lipids or carbohydrates. This increases their ability to adhere to teeth, either by prolonging the duration of the adhesion or increasing its affinity, or both This is particularly desirable for shorter  
15 polypeptides of the invention, which comprise up to around 40 amino acid residues.

Of the possible formulations, sterile pyrogen-free aqueous and non-aqueous solutions are preferred. Also preferred are formulations in which the polypeptides of the  
20 invention are contained in liposomes. Injection solutions and suspensions may be prepared extemporaneously from sterile powders, granules and tablets of the kind previously described.

Oral methods of administration may produce an effect  
25 systemically or locally in the mouth. Orally active preparations can be formulated in any suitable carrier, such as a gel, toothpaste, mouthwash or chewing gum.

It should be understood that in addition to the ingredients particularly mentioned above the formulations  
30 of this invention may include other agents conventional in the art having regard to the type of formulation in question.

Accordingly, the present invention provides a method of vaccinating a mammalian host against dental caries or

treating dental caries, which method comprises administering to the host an effective amount of a pharmaceutical composition as described above, for example a vaccine composition.

5       Antibodies, including monoclonal antibodies, can be formulated for passive immunisation as indicated above for the formulation of including polypeptides of the invention. Preferred formulations for passive immunisation include solid or liquid formulations such as gels, toothpastes,  
10   mouth-washes or chewing gum.

      A further aspect of the present invention is a naked nucleic acid vaccine. In this embodiment, the vaccine composition comprises a nucleic acid, typically an isolated nucleic acid, preferably DNA, rather than a polypeptide.  
15   The nucleic acid is injected in to a mammalian host and expressed in vivo, generating a polypeptide of the invention. This stimulates a T-cell response, which leads to protective immunity against dental caries in the same way as direct vaccination with a polypeptide of the  
20   invention.

      Naked nucleic acid vaccination can be carried out with any nucleic acid according to the invention as long as it encodes a polypeptide that stimulates a T-cell and or B-cell response. Preferred nucleic acids are those shown in  
25   SEQ. ID Nos. 1 to 11. These will typically be included within an expression vectors as defined above. In such an expression vector, the nucleic acid according to the invention will typically be operably linked to a promoter capable of directing its expression in a mammalian host  
30   cell. For example, promoters from viral genes that are expressed in the mammalian cells such as the cytomegalovirus (CMV) immediate early gene promoter are suitable. Also suitable are promoters from mammalian genes that are expressed in many or all mammalian cell types such

as the promoters of "housekeeping" genes. One such promoter is the p-hydroxymethyl-CoA-reductase (HMG) promoter (Gautier et al (1989): Nucleic Acids Research; 17,8839).

For naked nucleic acid vaccination, it is preferred  
5 that the nucleic acid sequence according to the invention is incorporated into a plasmid vector, since it has been found that covalent closed circle (CCC) plasmid DNA can be taken up directly by muscle cells and expressed without being integrated into the cells' genomic DNA (Ascadi et al  
10 (1991): The New Biologist; 3, 71-81). Naked nucleic acid vaccine may be prepared as any of the types of formulation mentioned above in respect of conventional polypeptide-based vaccines. However, formulations suitable for parenteral injection, especially intramuscular injection,  
15 are preferred. Naked nucleic acid vaccines may be delivered in any of the ways mentioned above in respect of conventional polypeptide-based vaccines but intramuscular injection is preferred.

Accordingly, the present invention provides a vaccine  
20 composition comprising a nucleic acid sequence or vector as described above and an acceptable carrier.

The following examples illustrate the invention.

## EXAMPLES

### 25 MATERIALS AND METHODS

**Materials.** Fmoc amino acids, benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) and Rink Amide MBHA resin were purchased from Calbiochem-Novabiochem (UK) Ltd., (Nottingham, UK). Dimethylformamide,  
30 trifluoroacetic acid, diethyl ether, dichloromethane and piperidine were purchased from Romil Chemicals Ltd (Loughborough, UK). Di-isopropylethylamine was from Aldrich Chemical Co. (Dorset, UK). Oligonucleotides were purchased from Oswel DNA Service (University of Edinburgh,



Edinburgh, UK).

#### Bacteria and growth conditions

- S. mutans* Guy's strain (serotype c) were grown in 10L basal medium supplemented as described previously (Russel et al (1978): Arch. Oral Biol., 23:17; Russel et al (1980): Infect. Immun. 61, 5490) at 37°C for 72h for SA I/II preparation. For the adhesion assay, *S. mutans* were grown in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.).
- Escherichia coli BL21 (DE3) (Novagen Inc., Madison, Wis.) harbouring pET15b were grown at 37°C in Luria-Bertani broth supplemented with carbenicillin (50µg/ml) and recombinant protein expression was induced with isopropyl-β-D-thiogalactopyranoside (1mM).
- Antigens.** SA I/II was prepared from *S. mutans* (serotype c, Guy's strain) as described by Russel et al (1980: Infect. Immun. 28, 486). Using the procedure of Munro et al (1993: Infect. Immun. 61, 4590), the portion of the gene encoding residues 984-1161 was amplified by using the oligonucleotide primers: (5') ATACATATGCCAACTGTTCATTTCCATTACTTT (SEQ. ID. No. 25) and (3') GCCATTGTCGACTCATTCAATTTTATTAACTTAGT (SEQ. ID. No. 26), cloned into pET15b (modified by the addition of a Sal I site) and expressed in *E. coli*.
- Synthetic peptides.** Peptide amides (20 mers overlapping by 10 residues) were synthesised on Rink amide MBHA resin in sealed porous polypropylene bags by the manual simultaneous multiple peptide synthesis procedure (Houghten (1985) PNAS 89:2, 5131) using Fmoc chemistry. PyBOP was used as coupling agent and Fmoc amino acids were activated in situ by addition of diisopropylethylamine. Following 20 cycles of synthesis, resin was washed with dimethylformamide followed by dichloromethane and peptides were cleaved by

incubation in trifluoroacetic acid-ethanedithiol-anisole-phenol-H<sub>2</sub>O (82.5:2.5:5:5:5; v/v/v/w/v) for 2h at room temperature. Peptides were precipitated by the addition of 5 volumes ether, recovered by centrifugation and washed  
5 three times with ether. Finally, peptides were dissolved in water and lyophilised. The scale of synthesis was 50µmol. Aliquots of each peptide were hydrolysed in 6M HCl at 110°C for 24h and compositions were determined using the Beckman 121MB automated analyser (Beckman Instruments Ltd,  
10 Bucks, UK). In each case the composition matched that predicted.

**Antibodies.** MAbs, L243 (anti-MHC class II) and W6/32 (anti-MHC class I) were produced from cultures of  
15 hybridomas obtained from the American Type Culture Collection (Rockville, Md, USA). ID4 an isotype (IgG2a) matched control of irrelevant specificity was provided by Dr. P. Shepherd (Department of Immunology, UMDS, Guys Hospital, London, UK). Rabbit anti-SA I/II antiserum was  
20 prepared as described previously (Russel et al (1980). Infect. Immun. 28, 486).

**Lymphoproliferative assay.** Defibrinated blood from volunteers was separated on a Ficoll gradient. Sera was  
25 used for antibody assays (see below) while peripheral blood mononuclear cells (PBMCs) were washed and resuspended in RPMI 1640 (Sigma Chemical Co., St. Louis, Mo, USA) supplemented with 2mM L-glutamine, penicillin (100 IU/ml), streptomycin sulphate (100 µg/ml) and 10% heat-inactivated  
30 autologous serum. PBMCs (10<sup>5</sup> cells/well) were cultured in 96-well round-bottomed plates (Costar, Cambridge, Mo, USA) in a total volume of 200 µl. Three replicates of each culture were incubated with three concentrations (1, 10 and 40 µg/ml) of SA I/II, recombinant fragments, non-

- recombinant control or synthetic peptides. Incubation was at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> for 6 days. Each culture received 0.2 µCi (7.4kBq) of [<sup>3</sup>H]-thymidine (Amersham International, Bucks, UK) 6h before harvesting.
- 5 Cultures were harvested onto glass fibre filters using a Dynatech (Chantilly, VA, USA) Minimal Cell harvester and [<sup>3</sup>H]-thymidine incorporation was measured using the LKB liquid scintillation counter (Bromma, Sweden).
- Proliferation was expressed as stimulation index which is
- 10 mean counts per minute (cpm) of antigen-stimulated, divided by cpm of antigen-free cultures. Concanavalin A (10 µg/ml) (Sigma Chemical Co., St. Louis, Mo, USA) was used with every culture as a positive control but the results are not presented.
- 15 MHC dependency of proliferative responses to SA I/II was determined by culturing cells with antigen (10 µg/ml) as above in the presence of MAbs L235, W6/32 or ID4 at 1, 10 and 20 µg/ml. Cultures were incubated with [<sup>3</sup>H]-thymidine, harvested and [<sup>3</sup>H]-thymidine uptake was
- 20 determined as described above.

- ELISA for serum antibodies.** Antibody recognition of synthetic peptides was determined by ELISA. Peptides (10 µg/ml) in phosphate buffered saline (PBS) were adsorbed to
- 25 wells of polystyrene microtitre plates (Dynatech) for 2h at room temperature. Plates were washed and wells were treated with 1.5% (w/v) bovine serum albumen (BSA) for 1h at room temperature to block unbound sites. After washing, bound peptides were incubated with serially diluted sera in
- 30 duplicate. Bound IgG antibodies were determined by incubation with alkaline phosphate conjugated-goat anti-human Ig (Sigma Chemical Co.) and subsequent reaction with paranitrophenyl phosphate (Sigma Chemical Co.). Plates were read at 405nm using the microplate reader model 450

(Bio-Rad). After initial screening, the assay was repeated at least 3 times with each serum using a restricted set of peptides. SA I/II (2  $\mu$ g/ml) was included in each assay as was an irrelevant peptide (HQAAMQIIRDIINEEAADWD (SEQ. ID. No. 27) derived from the sequence of SIV p27. Results are expressed as the highest dilution giving an absorbance  $\geq 0.2$ .

**Western blotting.** Serum antibody responses were also assayed by Western blotting using SA I/II, the recombinant polypeptides and a control fraction from E. coli BL21 harbouring non-recombinant pET15b. Purified antigens were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with gels of 10% acrylamide, by using a mini-gel system (Hoeffer Scientific Instruments, San Francisco, Co, USA). Proteins were transferred to nitrocellulose with a semi-dry blotter (Sartorius A.G., Gottingen, Germany). Nitrocellulose strips were blocked with 5% (wt/vol) nonfat milk powder 2.5% (wt/vol) BSA in Tris-HCl-buffered saline (pH 8.0) containing 0.05% (wt/vol) Tween 20. Strips were subsequently incubated with human sera (1 in 20 dilution) or rabbit anti-SA I/II antiserum ( $10^{-4}$  dilution) and bound antibody was visualised by using alkaline phosphatase-conjugated secondary antibody with 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium (Sigma Chemical Co.) as substrates. Each sera was assayed three times and responses were considered as positive if bands were visible in at least two assays.

**Bacterial adherence assay.** SA I/II mediated adherence of S. mutans (Guy's strain) to saliva was assayed by determining binding of [ $^3$ H]-thymidine labelled bacteria to saliva adsorbed to microtitre wells. Freshly collected human saliva from a single donor was clarified by

centrifugation for 10 min at 3000g, heat-inactivated at 60°C for 30 min and finally clarified by centrifugation at 17,000g for 20 min. Treated saliva was diluted with an equal volume of PBS and adsorbed to the wells of a

5 polystyrene 96-well flat-bottomed microtitre plate (Immulon 4; Dynatech) for 2h at room temperature. After coating, wells were washed three times with PBS and unbound sites were blocked by incubation with 1.5% (wt/vol) BSA in PBS for 1h at room temperature. Plates were then washed three

10 times with 50 mM KCl-1mM CaCl<sub>2</sub>-38 mM MgCl<sub>2</sub>-1 mM KH<sub>2</sub>PO<sub>4</sub>-1.2 mM K<sub>2</sub>PO<sub>4</sub> (pH 7.2; adherence buffer). *S. mutans* cells from an overnight culture in Todd-Hewitt broth were used to inoculate (1/10 volume) a further culture in Todd-Hewitt broth containing 100μCi (3.7 MBq) [<sup>3</sup>H]-thymidine (Amersham

15 International plc) per ml. Cells were harvested in late log phase (O.D. 700nm approximately 0.4) pelleted by centrifugation at 1000g for 10 min and washed three times in adherence buffer. The final suspension was vortexed with 0.5 volume glass beads to break up chains of cocci

20 which was monitored microscopically (Munro *et al* (1993): Infect. Immun. 61, 4590). Cells were resuspended to 5x10<sup>4</sup> c.p.m. per 50 μl and BSA was added to 1.5% (wt/vol). Specific activity of the washed *S. mutans* cells was estimated to be 1.3x10<sup>-3</sup> c.p.m. per cell (Munro *et al*

25 (1985): Infect. Immun. 61, 4590). In competitive inhibition of adherence, the various synthetic peptides were added to the wells (at final concentrations 62.5-500μM) in 50 μl adherence buffer containing 1.5% (wt/vol) BSA together with 50 μl radiolabelled *S. mutans* suspension.

30 Microtitre plates were incubated at 37°C for 2h with gentle shaking and subsequently were washed ten times with adherence buffer. Bound *S. mutans* cells were eluted with 1% (wt/vol) SDS and transferred to glass fibre filters by using the Micromate 196 cell harvester (Canberra Packard,

Berks, UK). Filters were counted using the Matrix 96 direct beta counter (Canberra Packard). Background binding was determined on wells to which no saliva was adsorbed. The percentage of binding of *S. mutans* to saliva was calculated by the formula  $[(\text{test c.p.m.}) - (\text{control c.p.m.}) / \text{total c.p.m.}] \times 100$ . Percent inhibition of adherence was calculated as  $[(\text{percent adherence without inhibitor} - \text{percent adherence with inhibitor}) / \text{percent adherence without inhibitor}] \times 100$ . For proteins, determinations of streptococcal adhesion were made in triplicate or quadruplicate at each protein concentration while for peptides, duplicate determinations were made. In each case the assay was performed at least three times.

#### 15 Statistics

The student's t test was used to analyse results.

#### Example 1

Preparation of a panel of overlapping synthetic peptides and analysis of their properties.

#### T cell epitope mapping

A panel of 32 overlapping synthetic peptides, spanning residues 803-1174 of SA I/II, was prepared, as described above (See Figure 1). Proliferative responses of PBMCs from 30 subjects were determined by stimulation with peptides (see Figure 2). All subjects responded to at least one peptide with a band range of 1-8 peptides, and a mean of 4.4 peptides. On the basis of frequency of response to each peptide ( $SI \geq 3.0$  c.p.m.  $> 500$ ) 3 immunodominant epitopes were identified; peptides 803-822, 975-994 and 985-1004, each yielding frequencies  $> 50\%$  (Fig. 1). Since most (13/15) subjects who responded to peptide 975-994 also responded to peptide 985-1004, it is probable

that a single T-cell epitope is present within residues 975-1004. Minor T cell epitopes were also identified within peptides 1005-1024, 1015-1034, 1085-1104 and 1115-1134 with frequencies >20% and some of the adjacent peptides may  
5 represent single T cell epitopes.

**MHC restriction of the lymphoproliferative responses (See Figure 3 and Table 2)**

HLA restriction of the T cell response was first  
10 studied by dose-dependant inhibition with MAb to HLA class I and II antigen (Fig. 2). The lymphoproliferative response was inhibited by 50% with 1 $\mu$ g of MAb to HLA class II (L243) and 10 $\mu$ g of the MAb inhibited 100% of the responses (from SI 10.0 $\pm$ 3.2 to SI 1.5 $\pm$ 0.4). Neither MAb to  
15 HLA class I (W6/32) nor the isotype control induced any inhibition of the lymphoproliferative response.

The HLA-DR of 17 subjects were determined and 6 of these were homozygous. The responses of the immunodominant and minor epitopes were then studied in the 6 DR homologous  
20 subjects (Table 2). Only peptide 975-994 appeared to be restricted by HLA-DR1. The other 6 peptides stimulated lymphocytes from HLA-DR1, 2 (except AA 1085-1104) and DR6 (except AA 803-822). DR5 was restricted by peptide 803-922, though the latter stimulated lymphocytes with DR1, 2  
25 and 3 antigens. Lymphocytes with DR3 or 4 antigen responded to 3 or 4 peptides. The results suggest that except for peptide 975-994, the remaining 6 peptides appear to be promiscuous as they stimulated lymphocytes with 3 to 5 HLA-DR antigens.

Table 1

DR	803-822	975-994	985-1004	1005-1024	1014-1034	1085-1104	1115-1134
1	<b>4.1±1.0</b>	<b>4.0±1.3</b>	<b>5.8±1.8</b>	<b>3.2±0.6</b>	<b>3.3±1.1</b>	<b>3.3±1.3</b>	<b>3.2±0.6</b>
2	<b>19.3±6.6</b>	<b>2.2±0.4</b>	<b>16.7±1.7</b>	<b>14.6±5.7</b>	<b>11.2±5.2</b>	<b>0.6±0.3</b>	<b>14.7±3.3</b>
3	<b>6.1±2.7</b>	<b>0.7±0.2</b>	<b>4.1±2.3</b>	<b>1.0±0.2</b>	<b>2.1±1.7</b>	<b>4.3±1.2</b>	<b>1.9±2.3</b>
4	<b>2.5±0.8</b>	<b>1.8±0.7</b>	<b>3.0±0.3</b>	<b>3.2±0.5</b>	<b>1.6±0.1</b>	<b>3.7±0.6</b>	<b>1.5±0.7</b>
5	<b>6.8±1.0</b>	<b>1.8±1.3</b>	<b>2.0±0.8</b>	<b>2.3±0.5</b>	<b>1.3±0.3</b>	<b>1.2±0.4</b>	<b>2.9±2.8</b>
6	<b>2.6±1.5</b>	<b>2.9±0.9</b>	<b>3.5±0.5</b>	<b>8.3±3.1</b>	<b>5.7±2.4</b>	<b>5.6±1.4</b>	<b>5.0±2.0</b>

Table 1 The relationship between HLA-DR1-6 and the T cell responses to 7 synthetic peptides.

S.I (fsem) values of subjects homozygous for DR are shown.  
Positive responses (S.I.>3.0,c.p.m.>500) are in bold.



**B cell epitope mapping (see Figure 4)**

Recognition of the recombinant fragments was assessed by Western blotting. Representative blots obtained with sera from 3 individuals are shown in Fig. 3 together with a positive control using rabbit anti-SA I/II antiserum. In panel a, SA I/II, and 984-1161 were recognised strongly. Rabbit anti-SA I/II antiserum used as a positive control (panel d) recognised recombinant 984-1161. The recombinant polypeptide corresponding to residues 984-1161 was also analysed. SA I/II was recognised by all subjects. B cell epitopes were mapped by ELISA using the panel of synthetic peptides. The panel of peptides was screened with sera from 22 individuals and 8 peptides which were recognised by more than one individual, together with one peptide which was not recognised, were selected for further analyses (Fig.5). SA I/II was recognised by all subjects with mean  $\log_2$  titre of  $7.6 \pm 1.2$ . Titres against peptides were lower, with only that against peptide 824-843 (mean  $\log_2$  titre  $4.7 \pm 1.1$ ) being significantly greater than the titre against the control SIV p27 peptide ( $t=7.28$   $p<0.01$ ). The proportion of significant titres ( $>\text{mean} + 2$  standard derivations) was also calculated (Fig. 5) and only peptide 824-843 showed high frequency (18/22). Indeed, an immunodominant B cell epitope is present within peptide 824-843, possibly shared with the overlapping peptide 834-853, while peptides 925-944, 1035-1054 and 1085-1104 constitute minor B cell epitopes. Despite the high frequency of responses to the recombinant polypeptide 984-1161 described above), a very low frequency of responses was observed to peptides within this region.

Saliva samples from the subjects were cultured to determine levels of S. mutans. In 66% of individuals S. mutans was detected (range  $10^3$ - $10^5$  colony forming units/ml). There was no correlation between S. mutans

levels and recognition of particular epitopes or titre against SA I/II.

#### Adhesion epitope mapping

- 5 Adherence of S. mutans to saliva-coated microtitre wells (a model of the tooth surface) was determined with [<sup>3</sup>H]-thymidine labelled S. mutans. The proportion of adhering bacteria was in the range 1-5%. In the absence of saliva, the proportion of adhering bacteria was <0.1%.
- 10 In a series of competitive inhibition assays, the panel of synthetic peptides was assayed for inhibition of adhesion of S. mutans to saliva-coated microtitre wells. Peptides 1005-1024, 1025-1044 and 1085-1104 consistently inhibited adhesion with maximal inhibition  $\geq 90\%$  at
- 15 concentrations of 500  $\mu$ M (Fig. 6). Adjacent peptides 1015-1034 and 1095-1114 showed more variable and lower inhibition, and may be part of the adhesion epitopes.

#### Example 2

- 20 Construction of an expression vector and expression of a recombinant polypeptide of the invention (SEQ. ID. No. 8). Using the oligonucleotide primers TAT CAT ATG CAA GAT CTT CCA ACA CCT CCA TCT ATA (5') (SEQ. ID. NO. 29) and GTC GAC TCA TAC CAA GAC AAA GGA AGT TGT (3') (SEQ. ID. No.30) the
- 25 portion of the SA I/II gene encoding residues 975-1044 (SEQ. ID. No.8) was amplified by polymerase chain reaction. The amplified gene fragment (with introduced Nde I and Sal I restriction enzyme sites) was cloned using the Ta cloning system and was subcloned into the plasmid pET15b. The
- 30 recombinant polypeptide was expressed in E. coli BL21 (DE3).

#### Example 3

Stimulation of an in vitro T-cell response by the

recombinant polypeptide (SEQ. ID. No. 8).

Peripheral blood lymphocytes from human volunteers were prepared as described above. Cells were incubated with purified recombinant polypeptide 975-1044 at concentrations  
5 of 40, 10 and 1 $\mu$ g/ml. Cells were also incubated with a protein fraction prepared in the same way from E. coli harbouring non-recombinant plasmid. Proliferative responses of 17 subjects were determined. Mean stimulation index ( $\pm$ sem) was 11.6 $\pm$ 2.3 compared with 2.4 $\pm$ 0.3 for the  
10 control. The frequency of subjects responding (i.e. those with stimulation index  $\geq$  control + 2SD) was 15/17.

#### Example 4

Immunisation of mice with the recombinant polypeptide (SEQ.  
15 ID. NO. 8) (See Figure 7)

i) Groups of mice (3-4 per group) were immunised with 975-1044 (SEQ. ID. No. 8) by two routes:

a) intraperitoneally with 50 $\mu$ g polypeptide in incomplete Freund's adjuvant with a boost after 4 weeks (also 50 $\mu$ g in  
20 incomplete Freund's adjuvant and intraperitoneally).

b) subcutaneously. A single immunisation with 50 $\mu$ g polypeptide in incomplete Freund's adjuvant.

ii) Draining lymph nodes were removed 10 to 14 days after immunisation, pooled and homogenised to give a single cell  
25 suspension in RPMI 1640 culture medium supplemented with 2mM glutamine, 1mM pyruvate, 50mM 2-mercaptoethanol, 100u/ml penicillin, 100 $\mu$ g/ml streptomycin, 100mM HEPES and 5% foetal calf serum. Cells (2x10<sup>5</sup>/well) were cultured with antigen and proliferation was measured by  
30 incorporation of [<sup>3</sup>H]-thymidine as described above.

Antigens were SA I/II recombinant polypeptides, peptides spanning residues 975-1044 and a control protein fraction from E. coli harbouring non-recombinant plasmid.

As in Fig 7, all mouse strains responded to SA I/II

and the recombinant polypeptide 975-1044 (SEQ. ID. No. 8). Positive responses to peptides were those of stimulation index  $\geq 3.0$  (cpm > 500). SJL mice responded to peptide 985-1004 and DBA/a mice responded to peptide 975-995 and 985-1004. For BALB/c mice, no significant responses to peptides were observed although the response to peptide 985-1004 was greater than responses to the remaining peptides.

iii) **Antibody recognition (See Table 2)**

10        Sera from mice immunised intraperitoneally with polypeptide 975-1044 recognised intact cells of *S. mutans*, intact SA I/II and recombinant 975-1044. Peptides 995-1014 and 1025-1044 were also recognised. The titre for each strain was as in Table 2, which shows log<sub>2</sub> titres where  
15        initial dilution was 1 in 50 (titre = 1).

Table 2  
Antibody recognition of S.mutans, SA I/II and peptides.

STRAIN	ANTIGEN									
	<u>S.mutans</u>	SA I/II	975-1044	NR	Control	PEPTIDES				
						975-994	985-1004	995-1014	1015-1034	1025-1044
SJL	4.0	4.0	10.3	-	-	-	-	8.7	1.0	5.7
DBA/1	3.0	2.7	10.7	-	0.7	0.7	0.7	4.7	2.0	7.3
BALB/C	2.0	2.8	10.7	-	-	-	-	5.7	3.0	4.7

Numbers in the table are log, titres (1=1:50)

**Example 5****Analysis of the interaction between streptococcal antigen  
5 I/II and salivary receptor using BIAcore****AIMS**

In this study, we have used surface plasmon resonance (spr) to analyse the interaction between purified SA I/II and  
10 whole human saliva or purified salivary receptor. In addition we have investigated the calcium dependence of binding, identified individual amino acid residues which may be involved in binding and determined the affinity of the interaction between SA I/II and salivary receptor.

15

**METHODS****Materials**

SA I/II and recombinant polypeptides were prepared as described above. Salivary receptor was prepared by  
20 absorption of whole saliva with intact cells of *S. mutans* (Lee et al (1989) Infect. Immun. 57:3306-3313). The cells were washed with KPBS (2.7 mM KCl, 137mM NaCl in 1.5mM  $\text{KH}_2\text{PO}_4$ , 6.5mM  $\text{Na}_2\text{HPO}_4$ , pH 7.2) and adsorbed material was eluted with 1mM EDTA in KPBS. Analysis of the purified  
25 material by polyacrylamide gel electrophoresis in the presence of Na dodecyl sulphate indicated the presence of components of  $\text{Mr} > 200,000$  and approximately 40,000. Peptides were prepared by the simultaneous multiple peptide synthesis procedure (Houghten (1985) Proc. Natl. Acad. Sci.  
30 USA 82: 5131-5135) as above. In addition, a series of peptides was synthesised corresponding to residues 1025-1044 in which each residue in turn was substituted by alanine.

**Binding Analyses**

Purified SA I/II or salivary receptor was immobilised on the sensor chip surface at a concentration of 100 µg/ml in 10mM Na formate pH 3.5 using the amine coupling kit

5 (Pharmacia Biosensor).

**i. Inhibition studies**

Binding of immobilised SA I/II to receptors in whole saliva was determined in the absence and presence of inhibitors (at varying concentrations). Inhibition by alanine-

10 substituted peptides was analysed at a peptide concentration of 50 µM. The running buffer was HEPES buffered saline (HBS) and the surface was regenerated with 100mM HCl.

**ii. Direct binding**

15 Purified salivary receptor was immobilised on the sensor chip and binding of SA I/II or purified recombinant polypeptide fragments was determined.

**RESULTS****20 i. Calcium dependency**

In separate determinations with whole saliva, binding to immobilised SA I/II varied from approximately 250 resonance units (RU) - 800 RU. In the presence of EDTA, binding was inhibited with maximal inhibition of 95% at a concentration  
25 of 10mM EDTA. Subsequent binding assays were performed in the presence of 5mM calcium.

**ii. Inhibition of binding**

Purified SA I/II or recombinant polypeptide fragments 1 (residues 39-481), 2 (residues 475-824), 3 (residues 816-  
30 1213), 4 (residues 1155- 1538) and recombinant 984-1161 were added to fluid phase saliva as competitive inhibitors at concentrations varying from 0-20µM. SA I/II inhibited binding most efficiently with approximately 90% inhibition

at a concentration of 6  $\mu$ M (Fig.8). Of the recombinant fragments, only fragment 3 and r984-1161 inhibited binding to salivary receptors to a significantly greater extent than the control (bovine serum albumin) with maximal inhibition of 65% and 50%, respectively (Fig. 8).

A panel of synthetic peptides (20mers overlapping by 10) spanning residues 803-1174 was assayed for inhibitory activity. Peptide 1025-1044 was the most effective inhibitor although 10-20 fold higher concentrations were required than for polypeptides (Fig.9). A panel of peptides in which each of the residues 1025-1044 in turn were substituted with alanine (alanine was substituted by serine where it occurred naturally) was also analysed for inhibitory activity. Substitution of Glu (1037) consistently abolished inhibition mediated by the peptide (Fig.10). Similarly, substitution of Gln 1025, Thr 1039, Phe 1041, Val 1042, Leu 1043 and Val 1044 reduced the inhibition of binding which was mediated by the peptide 1025-1044.

### iii. Direct Binding

For these analyses, purified salivary receptor was immobilised on the sensor chip and binding to fluid phase SA I/II or recombinant polypeptides was determined. At a concentration of approximately 5  $\mu$ M both SA I/II and recombinant SA I/II bound to salivary receptor in the range 500-600 RU (Fig.11). Binding of recombinant polypeptides was determined at a concentration of approximately 20 $\mu$ M and highest binding was obtained with fragment 3 (1256 RU) (Fig.11). Binding of other fragments although significantly greater than the myosin control was not greater than the bovine serum albumin control and thus does not appear to be specific. Addition of EDTA (10  $\mu$ M) in



this assay completely inhibited binding of fluid phase SA I/II.

Affinity and rate constants for the adhesion-receptor interaction were determined for SA I/II, recombinant SA I/II and fragment 3 (Table 3). The values indicate a low affinity interaction with a slow association rate constant and a relatively rapid dissociation constant.

#### Conclusions

These analyses confirm that residues 816-1213 of SA I/II form an adhesion binding region and that within this region, peptide 1025-1044 forms an adhesion epitope. We have now extended these findings by identifying specific residues which may be essential for binding to salivary receptor, namely residues 1025, 1037, 1039 and 1041-1045. The binding is EDTA sensitive and, under the assay conditions, is of relatively low affinity.

TABLE 3

		SA I/II	recomb.SA I/II	FRAG 3
$k_a$	( $M^{-1} s^{-1}$ )	n.d.	$20.9 \times 10^3$	$1.5 \times 10^3$
$k_d$	( $s^{-1}$ )	$2 \times 10^{-2}$	$4.2 \times 10^{-3}$	$8.1 \times 10^{-3}$
$K_A$	( $M^{-1}$ )	n.d.	$5.0 \times 10^6$	$0.2 \times 10^6$

n.d. not determined

**SEQUENCE INFORMATION**

As a result of the experiments detailed above, the following sequences have been identified as being of particular interest.

- (i) Residues 925 to 1114 (SEQ. ID. No. 1). This sequence comprises sequences (iv) and (v) below and includes 2 series of overlapping T-cell, B-cell and adhesion epitopes, a further B-cell epitope, a further T-cell epitope and an adhesion site.

SEQ. ID. No. 1:

TEKPLEPAPVEPSYEAETPPTPTPDQPEPNKPVEPTYEVIPTPPTDPVYQDLPTPPSI  
PTVHFHYFKLAVQPQVNKEIRNNNDVNIDRTLVAKQSVVKFQLKTADLPAGRDETTSFV  
LVDPLPSGYQFNPEATKAASPGFDVAYDNATNTVTFKATAATLATFNADLTksvatiyp  
TVVGQVLNDGATY

Its DNA sequence is (SEQ.ID.No. 12):

ACAGAAAAGCCGTTGGAGCCAGCACCTGTTGAGCCAAGCTATGAAGCAGAGCCAACGCCA  
CCGACACCAACACCAGATCAACCAGAACCAAACAACTGTTGAGCCAACCTTATGAGGTT  
ATTCCAACACCGCCGACTGATCCTGTTTATCAAGATCTTCCAACACCTCCATCTATACCA  
ACTGTTCAATTCCATTACTTTAAACTAGCTGTTTCAGCCGCAGGTTAACAAGAAATTAGA  
AACAATAACGATGTTAATATTGACAGAACTTTGGTGGCTAAACAATCTGTTGTTAAGTTC  
CAGCTGAAGACAGCAGATCTCCCTGCTGGACGTGATGAAACAACCTTCCTTTGTCTTGGTA  
GATCCCCTGCCATCTGGTTATCAATTTAATCCTGAAGCTACAAAAGCTGCCAGCCCTGGC  
TTTGATGTCGCTTATGATAATGCAACTAATACAGTCACCTTCAAGGCAACTGCAGCAACT  
TTGGCTACGTTTAAATGCTGATTTGACTAAGTCAGTGGCAACGATTTATCCAACAGTGGTC  
GGACAAGTTCTTAATGATGGCGCAACTTAT

(ii) Residues 1005 to 1044 (SEQ. ID. No. 2). This comprises

a T-cell epitope overlapping two adhesion sites.

SEQ. ID. No. 2

5 NNNDVNIDRTLVAQSVVKFQLKTADLPAGRDETTTSFVLV

Its DNA sequence is (SEQ. ID. No. 13):

AACAATAACGATGTTAATATTGACAGAACTTTGGTGGCTAAACAATCTGTTGTTAAGTTC  
CAGCTGAAGACAGCAGATCTCCCTGCTGGACGTGATGAAACAACCTTCCTTTGTCTTGGA

10

(iii) Residues 1085-1104 (SEQ. ID. No. 3). Here, a T-cell  
epitope, a B-cell epitope and an adhesion site  
overlap.

SEQ. ID. No. 3:

15 LATFNADLTKSVATIYPTVV

Its DNA sequence is (SEQ. ID. No. 14):

TTGGCTACGTTTAATGCTGATTTGACTAAGTCAGTGGCAACGATTTATCCAACAGTGGTC

20 (iv) Residues 1005 to 1114 (SEQ. ID. No. 4). This comprises  
sequences (ii) and (iii) above and therefore includes  
two sequences in which a B-cell epitope a T-cell  
epitopes and an adhesion site overlap.

25 SEQ. ID. No. 4

NNNDVNIDRTLVAQSVVKFQLKTADLPAGRDETTTSFVLVDPLPSGYQFNPEATKAASPGF  
DVAYDNATNTVTFKATAATLATFNADLTKSVATIYPTVVVGQVLNDGATY

30 Its DNA sequence is (SEQ. ID. No. 15):

AACAATAACGATGTTAATATTGACAGAACTTTGGTGGCTAAACAATCTGTTGTTAAGTTC  
CAGCTGAAGACAGCAGATCTCCCTGCTGGACGTGATGAAACAACCTTCCTTTGTCTTGGA

GATCCCCTGCCATCTGGTTATCAATTTAATCCTGAAGCTACAAAAGCTGCCAGCCCTGGC  
TTTGATGTCGCTTATGATAATGCAACTAATACAGTCACCTTCAAGGCAACTGCAGCAACT  
TTGGCTACGTTTAATGCTGATTTGACTAAGTCAGTGGCAACGATTTATCCAACAGTGGTC  
GGACAAGTTCCTTAATGATGGCGCAACTTAT

5

(v) Residues 925 to 1004 (SEQ. ID. No. 5). This comprises  
a B-cell epitope, an immunodominant T-cell epitope and  
an adhesion site.

10 SEQ. ID. No. 5:

TEKPLEPAPVEPSYEAETPPTPTPDQPEPNKPVEPTYEVIPTPPTDPVYQDLPTPPSIPT  
VHFHYFKLAVQPQVNKEIR

15 Its DNA sequence is (SEQ. ID. No. 16):

ACAGAAAAGCCGTTGGAGCCAGCACCTGTTGAGCCAAGCTATGAAGCAGAGCCAACGCCA  
CCGACACCAACACCAGATCAACCAGAACCAAACAAACCTGTTGAGCCAACTTATGAGGTT  
ATTCCAACACCGCCGACTGATCCTGTTTATCAAGATCTTCCAACACCTCCATCTATACCA  
20 ACTGTTTCATTTCATTACTTTAACTAGCTGTTTCAGCCGCAGGTTAACAAAGAAATTAGA

(vi) Residues 925 to 1054 (SEQ. ID. No. 6). This comprises  
sequence (v) above, together with a further adjacent  
adhesion site and a further overlapping B-cell epitope.

25

SEQ ID. No. 6

TEKPLEPAPVEPSYEAETPPTPTPDQPEPNKPVEPTYEVIPTPPTDPVYQDLPTPPSIPT  
VHFHYFKLAVQPQVNKEIRNNNDVNIDRTLVAKQSVVKFQLKTADLPAGRDETTSFVLVDP

30 LPSGYQFN

Its DNA sequence is (SEQ. ID. No. 17):

ACAGAAAAGCCGTTGGAGCCAGCACCTGTTGAGCCAAGCTATGAAGCAGAGCCAACGCCA  
CCGACACCAACACCAGATCAACCAGAACCAAACAAACCTGTTGAGCCAACTTATGAGGTT  
ATTCCAACACCGCCGACTGATCCTGTTTATCAAGATCTTCCAACACCTCCATCTATACCA  
ACTGTTCATTTCCATTACTTTAACTAGCTGTTGAGCCGAGGTTAACAAAGAAATTAGA  
5 AACATAACGATGTTAATATTGACAGAACTTTGGTGGCTAAACAATCTGTTGTAAAGTTC  
CAGCTGAAGACAGCAGATCTCCCTGCTGGACGTGATGAAACAACCTTCCTTTGTCTTGGTA  
  
GATCCCCTGCCATCTGGTTATCAATTTAAT

- 10 (vii) Residues 803-854 (SEQ. ID. No. 7). This comprises a  
major T-cell epitope and adjacent immunodominant B-  
cell epitope.

SEQ. ID. No. 7:

15

ETGKKPNIWYSLNGKIRAVNLPKVTKEKPTPPVKPTAPTKPTYETEKPLKPA

Its DNA sequence is (SEQ. ID. No. 18)

20 GAAACCGGCAAAAAACCAAATATTTGGTATTCATTAAATGGTAAAATCCGTGCGGTTAAT  
CTTCCTAAAGTTACTAAGGAAAAACCCACACCTCCGGTTAAACCAACAGCTCCAACATAA  
CCAACTTATGAAACAGAAAAGCCATTAAAACCGGCA

- 25 (viii) Residues 975 to 1044 (SEQ. ID. No. 8). This  
comprises a T-cell epitope, a B-cell epitope and an  
adhesion site.

SEQ. ID No. 8:

30 QDLPTPPSIPTVHFHYFKLAVQPQVNKEIRNNNDVNIDRTLVAKQSVVKFQLKTADLPAGR  
DETTSFVLV

Its DNA sequence is (SEQ. ID. No. 19):

CAAGATCTTCCAACACCTCCATCTATACCAACTGTTTCATTTCCATTACTTTAAACTAGCT  
GTTTCAGCCGCAGGTTAACAAGAAATTAGAAACAATAACGATGTTAATATTGACAGAACT  
TTGGTGGCTAAACAATCTGTTGTTAAGTTCCAGCTGAAGACAGCAGATCTCCCTGCTGGA  
CGTGATGAAACAACTTCCTTTGTCTTGGA

5

(ix) Residues 1024 to 1044 (SEQ. ID. No. 9). This comprises  
a T-cell epitope overlapping with an adhesion site.

SEQ. ID. No. 9

10 FQLKTADLPAGRDETTSFVLV

Its DNA Sequence is (SEQ. ID. No. 20):

TTCCAGCTGAAGACAGCAGATCTCCCTGCTGGACGTGATGAAACAACTTCCTTTGTCTTG  
15 GTA

(x) Residues 803 to 1114 (SEQ. ID. No. 10). This comprises  
sequences (i) and (vii) above and some intervening  
sequence. Residues 803 to 1114 comprise 2 series of  
20 overlapping T-cell, B-cell and adhesion epitopes, a  
further T-cell epitope and a further adhesion site and  
an immunodominant B-cell epitope and a major T-cell  
epitope.

25 SEQ. ID. No. 10:

ETGKKPNIWYSLNGKIRAVNLPKVTKEKPTPPVKPTAPT KPTYETEKPLKPAPV  
APNYEKEPTPPTRTPDQAEPPKKPTPPTYETEKPLEPAPVEPSYEA EPTPPTRTPDQAE  
PNKPTPPTYETEKPLEPAPVEPSYEA EPTPPTPTPDQPEPNKPVEPTYEVIPTPPTDP  
VYQDLPTPPSIPTVHFHYFKLAVQPQVNKEIRNNNDVNIDRTLVAQSVVKFQLKTAD  
30 LPAGRDETTSFVLVDPLPSGYQFNPEATKAASPGFDVAYDNATNTVTFKATAATLATF  
NADLT KSVATIYPTVVGQVLNDGATY

Its DNA Sequence is (SEQ. ID. NO. 21):

GAAACCGGCACAAAAACCAAATATTTGGTATTCATTAAATGGTAAAATCCGTGCGGTTAAT  
CTTCCTAAAGTTACTAAGGAAAAACCCACACCTCCGGTTAAACCAACAGCTCCAACTAAA  
CCAACCTTATGAAACAGAAAAGCCATTAAACCGGCACCAGTAGCTCCAAATTATGAAAAG  
GAGCCAACACCACCGACAAGAACACCGGATCAAGCAGAGCCAAAGAAACCCACTCCGCCG  
5 ACCTATGAAACAGAAAAGCCGTTGGAGCCAGCACCTGTTGAGCCAAGCTATGAAGCAGAG  
CCAACACCGCCGACAAGGACACCGGATCAGGCAGAGCCAAATAAACCCACACCGCCGACC  
TATGAAACAGAAAAGCCGTTGGAGCCAGCACCTGTTGAGCCAAGCTATGAAGCAGAGCCA  
ACGCCACCGACACCAACACCAGATCAACCAGAACCAAAACAAACCTGTTGAGCCAACCTTAT  
GAGGTTATTCCAACACCGCCGACTGATCCTGTTTATCAAGATCTTCCAACACCTCCATCT  
10 ATACCAACTGTTCAATTTCCATTACTTTAAACTAGCTGTTGAGCCGAGGTTAACAAGAA  
ATTAGAAACAATAACGATGTTAATATTGACAGAACTTTGGTGGCTAAACAATCTGTTGTT  
AAGTTCCAGCTGAAGACAGCAGATCTCCCTGCTGGACGTGATGAAACAACCTTCCTTTGTC  
TTGGTAGATCCCCTGCCATCTGGTTATCAATTTAATCCTGAAGCTACAAAAGCTGCCAGC  
CCTGGCTTTGATGTCGCTTATGATAATGCAACTAATACAGTCACCTTCAAGGCAACTGCA  
15 GCAACTTTGGCTACGTTTAAATGCTGATTTGACTAAGTCAGTGGCAACGATTTATCC/ACA  
GTGGTCGGACAAGTTCCTTAATGATGGCGCAACTTAT

(xi) Residues 975 to 1004 (SEQ. ID. No. 11), which comprise  
a T-cell epitope.

20

SEQ. ID. No. 11:

QDLPTPPSIPTVHFHYFKLAVQPQVNKEIR

Its DNA Sequence is (SEQ. ID. NO. 22):

25 CAAGATCTTCCAACACCTCCATCTATACCAACTGTTCAATTTCCATTACTTTAAACTAGCT  
GTTGAGCCGAGGTTAACAAGAAATTAGA

The amino acid sequence of SA I/II is as follows, beginning  
with residue No. 1 (SEQ ID No. 23).

30

MKVKKTYGFRKSKISKTLGAVLGTVAAVSVAGQKVFADETTTT  
SDVDTKVVGTTQGNPATNLPEAQGSASKQAEQSQTKLERQMVHTIEVPKTDLDQAAKD  
AKSAGVNVVQDADVNKGTVKTAEEAVQKETEIKEDYTKQAEDIKKTDDQYKSDVAAHE

AEVAKIKAKNQATKEQYGKDMVAHKAEVERINAANAASKTAYEAKLAQYQADLAAVQK  
 TNAANQASYQKALAAQYQAE LKRVQEANAAKAAAYDTAVAANNAKNTETIAAANEEIRKR  
 NATAKAEYETKLAQYQAE LKRVQEANAAEADYQAKLTAYQTE LARVQKANADAKAAAY  
 EAAVAANNAKNAALTAENTA IKORNENAKATYEAALKQYEADLAAVKKANAANEADYQ  
 5 AKLTAYQTE LARVQKANADAKAAAYEAAVAANNAANAALTAENTA IKRNADAKADYEA  
 KLAKYQADLAKYQKDLADYPVKLKAYEDEQASIKAAALAELEKHKNE DGNLTETPSAQN  
 VYDLEPNANLSLTDDGKFLKASAVDDAFSKSTSKAKYDQKILQLDDLDITNLEQSN  
 ASSMELYGNFGDKAGWSTTVSNNSQVKGSVLLERGSATATYTNLQNSYNGKKISK  
 IVYKYTVDPKSKFQGGQKVLGIFTDPTLGVFASAYTGQVEKNTSIFIKNEFTFYDE  
 10 KPINF DNALLSVASLNRENNSIEMAKDYTGKFKISGSSIGEKNGMIYATDTLNFRQ  
 QGGARWTMYTRASEPGSGWDSSDAPNSWYGAGAIRMSGPNNSVTLGAISSTLVVPADP  
 TMAIETGKKPNIWYSLNGKIRAVNLPKVTKEKPTPPVKPTAPT KPTYETEKPLKPAPV  
 APNYEKEPTPPTRTPDQAE PKKPTPPTYETEKPLEPAPVEPSYEA EPTPPTRTPDQAE  
 PNKPTPPTYETEKPLEPAPVEPSYEA EPTPPTPTPDQPEPNKPVEPTYEVIPTPTDF  
 15 VYQDLPTPPSIPTVHFHYFKLAVQPQVNKEIRNNNDVNIDRTLVAQSVVKFQLKTAD  
 LPAGRDETTSFVLVDPLPSGYQFNPEATKAASPGFDVAYDNATNTVTFKATAATLATF  
 NADLT KSVATIYPTVVGQVLNDGATYKNNFSLTVNDAYGIKSNVVRVTTPGKPNDPDN  
 PNNNYIKPTKVNKNENG VVIDGKTVLAGSTNYEELTWDL DQYKNDRSSADTIQOGFY  
 VDDYPEEAELELRQDLVKITDANGNEVTGVSVDNYTSLEAAPQEIRDVLSKAGIRPKGA  
 20 FQIFRADNPREFYDTYVKTGIDLKIVSPMVVKQMGQTGGSYEDQAYQIDFGNGYASN  
 IVINNVPKINPKKDVTLTLD PADTNNDVGQTIPLNTVFNRYRLIGGIIPANHSEELFEY  
 NFDYDDYDQTDGHTGQYKVF AKVDITLKNGVIIKSGTELTQYTTAEVDTTKGAITIKF  
 KEAFLRSVSIDSAFQAESYIQMKRIA VGTGFENTYINTVNGVTYSSNTVKTTTTPEDPAD  
 PTDPQDPSSPRTSTV IYKQSTAYQPSSVQKTL PNTGVTNNAYMPLLGIIGLVTSFSL  
 25 LGLKAKKD

Its DNA sequence is as follows (SEQ ID No. 24):

1 ATTT CAGCAA AAATTGACAA ATCAAATCAA TTATATTACA ATTTTTTAAC  
 30 51 GTATATTACA AAAATATATT TGGAAGATTT ATTCAGATTT GGAGGATTTA  
 101 TGAAAGTCAA AAAA ACTTAC GGTTTTCGTA AAAGTAAAT TAGTAAACA  
 151 CTGTGTGGTG CTGTTCTAGG AACAGTAGCA GCAGTCTCTG TAGCAGGACA  
 201 AAAGGTTTTT GCCGATGAAA CGACCACTAC TAGTGATGTA GATACTAAAG



251 TAGTTGGAAC ACAAACTGGA AATCCAGCGA CCAATTTGCC AGAGGCTCAA  
301 GGAAGTGCGA GTAAGCAAGC TGAACAAAGT CAAACCAAGC TGGAGAGACA  
351 AATGGTTCAT ACCATTGAAG TACCTAAAAC TGATCTTGAT CAAGCAGCAA  
401 AAGATGCTAA GTCTGCTGGT GTCAATGTTG TCCAAGATGC CGATGTTAAT  
5 451 AAAGGAACTG TTAAAACAGC TGAAGAAGCA GTCCAAAAAG AACTGAAAT  
501 TAAAGAAGAT TACACAAAAC AAGCTGAGGA TATTAAGAAG ACAACAGATC  
551 AATATAAATC GGATGTAGCT GCTCATGAGG CAGAAGTTGC TAAAATCAAA  
601 GCTAAAAATC AGGCAACTAA AGAACAGTAT GGAAAAGATA TGGTAGCTCA  
651 TAAAGCCGAG GTTGAACGCA TTAATGCTGC AAATGCTGCC AGTAAAACAG  
10 701 CTTATGAAGC TAAATTGGCT CAATATCAAG CAGATTTAGC AGCCGTTCAA  
751 AAAACCAATG CTGCCAATCA AGCATCCTAT CAAAAGCCC TTGCTGCTTA  
801 TCAGGCTGAA CTGAAACGTG TTCAGGAAGC TAATGCAGCC GCCAAGCCG  
851 CTTATGATAC TGCTGTAGCA GCAAATAATG CAAAAATAC AGAAATTGCC  
901 GCTGCCAATG AAGAAATTAG AAAACGCAAT GCAACGGCCA AAGCTGAATA  
15 951 TGAGACTAAG TTAGCTCAAT ATCAAGCTGA ACTAAAGCGT GTTCAGGAAG  
1001 CTAATGCCGC AAACGAAGCA GACTATCAAG CTAAATTGAC CGCCTATCAA  
1051 ACAGAGCTTG CTCGCGTTCA GAAAGCCAAT GCAGATGCTA AAGCGGCTA  
1101 TGAAGCAGCT GTAGCAGCAA ATAATGCCAA AAATGCGGCA CTTACAGCTG  
1151 AAAATACTGC AATTAAGCAA CGCAATGAGA ATGCTAAGGC GACTTATGAA  
20 1201 GCTGCACTCA AGCAATATGA GGCTGATTTG GCAGCGGTGA AAAAGCTAA  
1251 TGCCGCAAAC GAAGCAGACT ATCAAGCTAA ATTGACCGCC TATCAAACAG  
1301 AGCTCGCTCG CGTTCAAAAG GCCAATGCGG ATGCTAAAGC GGCCTATGAA  
1351 GCAGCTGTAG CAGCAAATAA TGCCGCAAAT GCAGCGCTCA CAGCTGAAAA  
1401 TACTGCAATT AAGAAGCGCA ATGCGGATGC TAAAGCTGAT TACGAAGCAA  
25 1451 AACTTGCTAA GTATCAAGCA GATCTTGCCA AATATCAAAA AGATTTAGCA  
1501 GACTATCCAG TTAAGTTAAA GGCATACGAA GATGAACAAG CTTCTATTAA  
1551 AGCTGCACTG GCAGAACTTG AAAACATAA AAATGAAGAC GGAAACTTAA  
1601 CAGAACCATC TGCTCAAAAT TTGGTCTATG ATCTTGAGCC AAATGCGAAC  
1651 TTATCTTTGA CAACAGATGG GAAGTTCCTT AAGGCTTCTG CTGTGGATGA  
30 1701 TGCTTTTAGC AAAAGCACTT CAAAAGCAAA ATATGACCAA AAAATTCTTC  
1751 AATTAGATGA TCTAGATATC ACTAACTTAG AACAATCTAA TGATGTTGCT  
1801 TCTTCTATGG AGCTTTATGG CAATTTTGGT GATAAAGCTG GCTGGTCAAC  
1851 GACAGTAAGC AATAACTCAC AGGTAAATG GGGATCGGTA CTTTTAGAGC

1901 GCGGTCAAAG CGCAACAGCT ACATACACTA ACCTGCAGAA TTCTTATTAC  
2001 GTCCAAGTTT CAAGGTCAAA AGGTTTGGTT AGGTATTTTT ACCGATCCAA  
1951 AATGGTAAAA AGATTTCTAA AATTGTCTAC AAGTATACAG TGGACCCTAA  
2051 CTTTAGGTGT TTTTGCTTCC GCTTATACAG GTCAAGTTGA AAAAAACACT  
2101 TCTATTTTTTA TTAAAAATGA ATTCACTTTC TATGACGAAG ATGGAAAACC  
2151 AATTAATTTT GATAATGCCC TTCTATCAGT AGCTTCTCTT AACCGAGAAA  
2201 ATAATTCTAT TGAGATGGCC AAAGATTATA CGGGTAAATT TGTCAAAATC  
2251 TCTGGATCAT CTATCGGTGA AAAGAATGGC ATGATTTATG CTACAGATAC  
2301 TCTCAACTTT AGGCAGGGTC AAGGTGGTGC TCGTTGGACC ATGTATACCA  
10 2351 GAGCTAGCGA ACCGGGATCT GGCTGGGATA GTTCAGATGC GCCTAACTCT  
2401 TGGTATGGTG CTGGTGCTAT CCGCATGTCT GGTCCTAATA ACAGTGTGAC  
2451 TTTGGGTGCT ATCTCATCAA CACTTGTTGT GCCTGCTGAT CCTACAATGG  
2501 CAATTGAAAC CGGCAAAAAA CCAAATATTT GGTATTCATT AAATGGTAAA  
2551 ATCCGTGCGG TTAATCTTCC TAAAGTTACT AAGGAAAAAC CCACACCTCC  
15 2601 GGTAAACCA ACAGCTCCAA CTAAACCAAC TTATGAAACA GAAAAGCCAT  
2651 TAAACCGGC ACCAGTAGCT CCAAATTATG AAAAGGAGCC AACACCACCG  
2701 ACAAGAACAC CGGATCAAGC AGAGCCAAAG AAACCCACTC CGCCGACCTA  
2751 TGAAACAGAA AAGCCGTTGG AGCCAGCACC TGTGAGCCA AGCTATGAAG  
2801 CAGAGCCAAC ACCGCCGACA AGGACACCGG ATCAGGCAGA GCCAAATAAA  
20 2851 CCCACACCGC CGACCTATGA AACAGAAAAG CCGTTGGAGC CAGCACCTGT  
2901 TGAGCCAAGC TATGAAGCAG AGCCAACGCC ACCGACACCA ACACCAGATC  
2951 AACCAGAACC AAACAAACCT GTTGAGCCAA CTTATGAGGT TATTCACA  
3001 CCGCCGACTG ATCCTGTTTA TCAAGATCTT CCAACACCTC CATCTATACC  
3051 AACTGTTTCT TTCCATTACT TTAAACTAGC TGTTTCAGCCG CAGGTTAACA  
25 3101 AAGAAATTAG AAACAATAAC GATGTTAATA TTGACAGAAC TTTGGTGGCT  
3151 AAACAATCTG TTGTTAAGTT CCAGCTGAAG ACAGCAGATC TCCCTGCTGG  
3201 ACGTGATGAA ACAACTTCCT TTGTCTTGGT AGATCCCCTG CCATCTGGTT  
3251 ATCAATTTAA TCCTGAAGCT ACAAAGCTG CCAGCCCTGG CTTTGATGTC  
3301 GCTTATGATA ATGCAACTAA TACAGTCACC TTCAAGGCAA CTGCAGCAAC  
30 3351 TTTGGCTACG TTTAATGCTG ATTTGACTAA GTCAGTGGCA ACGATTTATC  
3401 CAACAGTGGT CGGACAAGTT CTTAATGATG GCGCAACTTA TAAGAATAAT  
3451 TTCTCGCTCA CAGTCAATGA TGCTTATGGC ATTAAATCCA ATGTTGTTTCG  
3501 GGTGACAACT CCTGGTAAAC CAAATGATCC AGATAACCCA AATAATAATT

3551 ACATTAAGCC AACTAAGGTT AATAAAAATG AAAATGGCGT TGTTATTGAT  
3601 GGTA AACAG TTCTTGCCGG TTCAACGAAT TATTATGAGC TAACTTGGGA  
3651 TTTGGATCAA TATAAAAACG ACCGCTCTTC AGCAGATACC ATTCAACAAG  
3701 GATTTTACTA TG TAGATGAT TATCCAGAAG AAGCGCTTGA ATTGCGTCAG  
5 3751 GATTTAGTGA AGATTACAGA TGCTAATGGC AATGAAGTTA CTGGTGTTAG  
3801 TGTGGATAAT TATACTAGTC TTGAAGCAGC CCCTCAAGAA ATTAGAGATG  
3851 TTCTTTCTAA GGCAGGAATT AGACCTAAAG GTGCTTTCCA AATTTTCCGT  
3901 GCCGATAATC CAAGAGAATT TTATGATACT TATGTCAAAA CTGGAATTGA  
3951 TTTGAAGATT GTATCACCAA TGGTTGTTAA AAAACAAATG GGACAAACAG  
10 4001 GCGGGAGTTA TGAAGATCAA GCTTACCAAA TTGACTTTGG TAATGGTTAT  
4051 GCATCAAATA TCGTTATCAA TAATGTTCCCT AAGATTAACC CTAAGAAAGA  
4101 TGTGACCTTA ACACTTGATC CGGCTGATAC AAATAATGTT GATGGTCAGA  
4151 CTATTCCACT TAATACAGTC TTTAATTACC GTTTGATTGG TGGCATTATC  
4201 CCTGCAAATC ACTCAGAAGA ACTCTTTGAA TACAATTTCT ATGATGATTA  
15 4251 TGATCAAACA GGAGATCACT ATACTGGTCA GTATAAAGTT TTTGCCAAGG  
4301 TTGATATCAC TCTTAAAAAC GGTGTTATTA TCAAGTCAGG TACTGAGITA  
4351 ACTCAGTATA CGACAGCGGA AGTTGATACC ACTAAAGGTG CTATCACAAT  
4401 TAAGTTCAAG GAAGCCTTTC TGC GTTCTGT TTCAATTGAT TCAGCCTTCC  
4451 AAGCTGAAAAG TTATATCCAA ATGAAACGTA TTGCGGTTGG TACTTTTGAA  
20 4501 AATACCTATA TTAATACTGT CAATGGGGTA ACTTACAGTT CAAATACAGT  
4551 GAAAACAACT ACTCCTGAGG ATCCTGCAGA CCCTACTGAT CCGCAAGATC  
4601 CATCATCACC GCGGACTTCA ACTGTAATTA TCTACAAACC TCAATCAACT  
4651 GCTTATCAAC CAAGCTCTGT CCAAAAAACG TTACCAAATA CGGGAGTAAC  
4701 AAACAATGCT TATATGCCTT TACTTGGTAT TATTGGCTTA GTTACTAGTT  
25 4751 TTAGTTTGCT TGGCTTAAAG GCTAAGAAAG ATTGACAGCA TAGATATTAC  
4801 ATTAGAATTA AAAAGTGAGA TGAAGCGATA AATCACAGAT TGAGCTTTTA  
4851 TCTCATTTTT TGATT

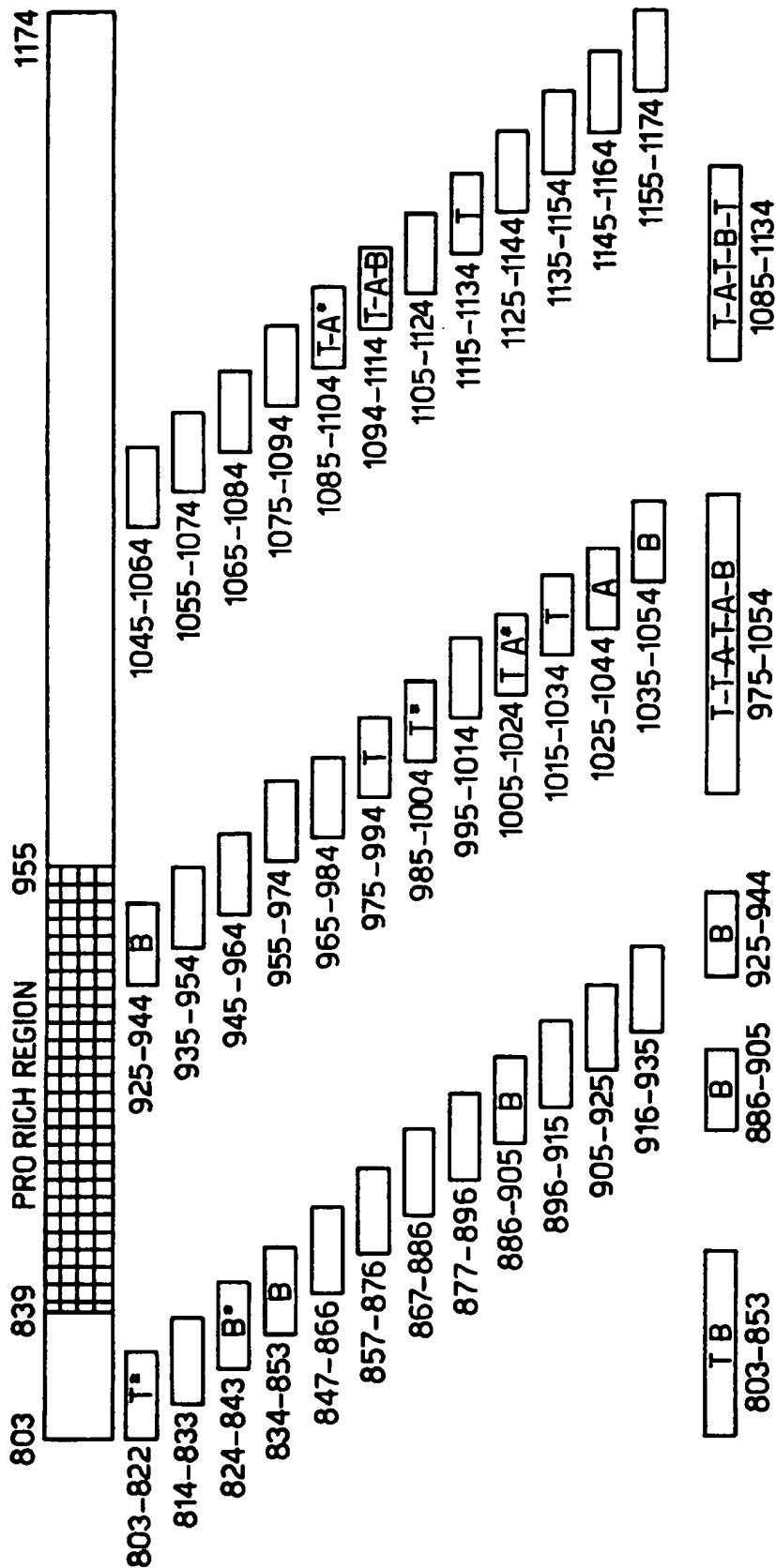
## CLAIMS

1. A nucleic acid sequence which codes upon expression in  
5 a prokaryotic or eukaryotic host cell for a polypeptide  
product having one or more properties selected from (i) the  
ability to adhere to a mammalian tooth in a competitive  
manner with naturally occurring Streptococcus mutans antigen  
I/II, thus preventing or diminishing the adhesion of S.mutans  
10 to the tooth; (ii) the ability to stimulate a T-cell  
response; and (iii) the ability to stimulate a B-cell  
response, said nucleic acid sequence being selected from:  
(a) the sequences shown in SEQ. ID. No. 12 to 22 or the  
complementary strands thereof;  
15 (b) nucleic acid sequences having a length of not more  
than 1000 base pairs which hybridise to the sequences  
defined in (a) over at least 70% of their length;  
(c) nucleic acid sequences having a length of not more  
20 than 1000 base pairs which, but for the degeneracy of  
the genetic code, would hybridise to the nucleic acid  
sequences defined in (a) or (b) over at least 70% of  
their length and which sequences code for polypeptides  
having the same amino acid sequence.
- 25 2. A nucleic acid sequence as defined in claim 1 which is  
extended at either or both of the 5' and 3' ends by non-wild-  
type nucleic acid sequence.
- 30 3. A polypeptide encoded by a nucleic acid sequence as  
defined in claim 1 or 2.
4. A polypeptide according to claim 3 comprising a

sequence as shown in any one of SEQ. ID. No. 1 to 11.

5. A vector comprising a nucleic acid sequence according  
5 to claim 1 or 2 operably linked to a promoter capable of  
directing its expression in a host cell.
6. A Cell harbouring a vector according to claim 5.
- 10 7. A pharmaceutical composition comprising a polypeptide  
according to claim 3 or 4 and a pharmaceutically acceptable  
carrier.
8. A composition comprising according to claim 7 that is a  
15 vaccine composition.
9. A polypeptide according to claim 3 or 4 for use in the  
treatment of dental caries.
- 20 10. A method of vaccinating a mammalian host against dental  
caries or treating dental caries, which method comprises  
administering to the host an effective amount of a  
polypeptide according to claim 3 or 4 or a composition  
according to claim 8 or 9.

Fig.1.



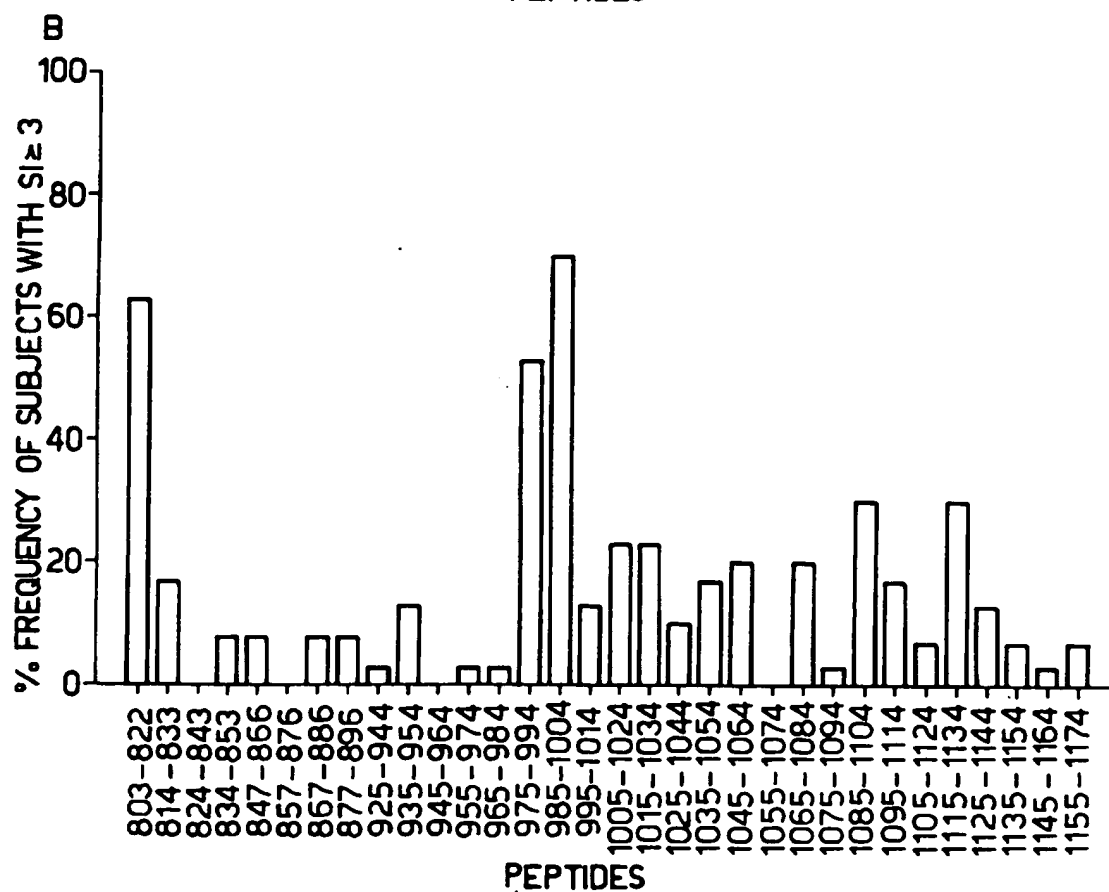
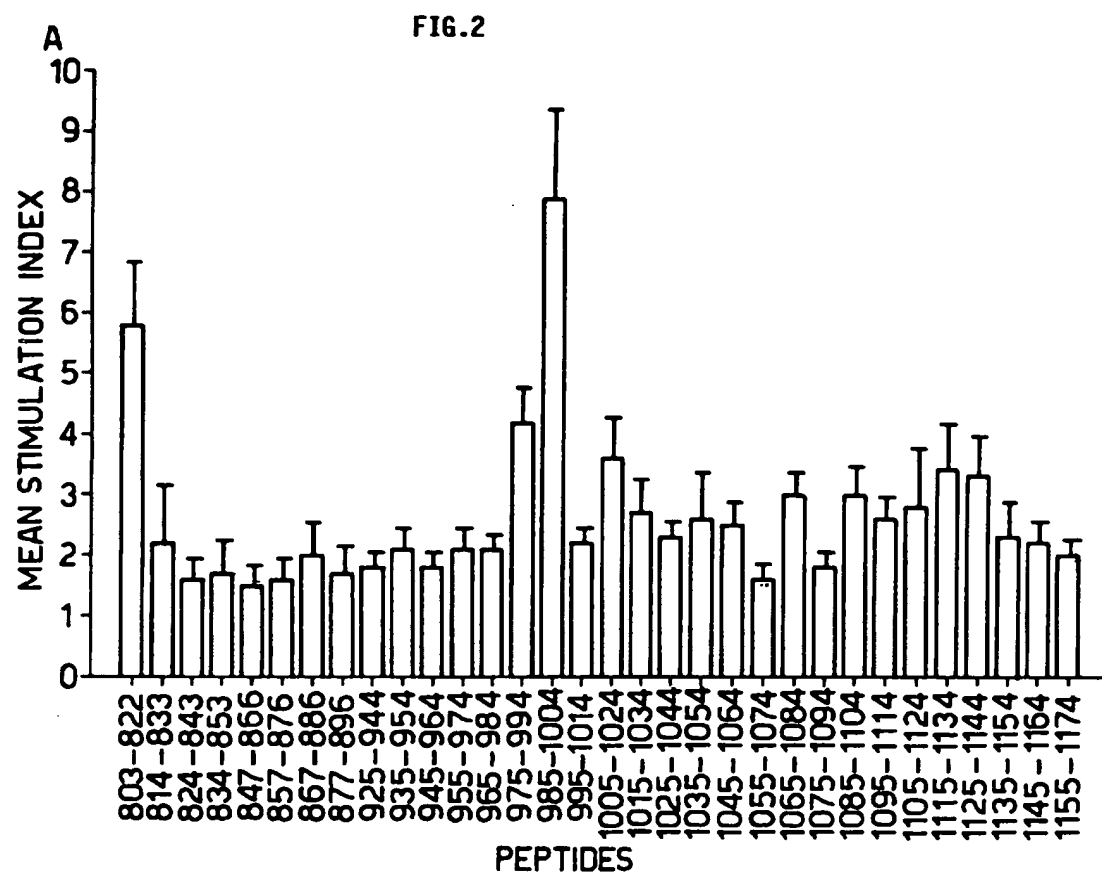


Fig.3.

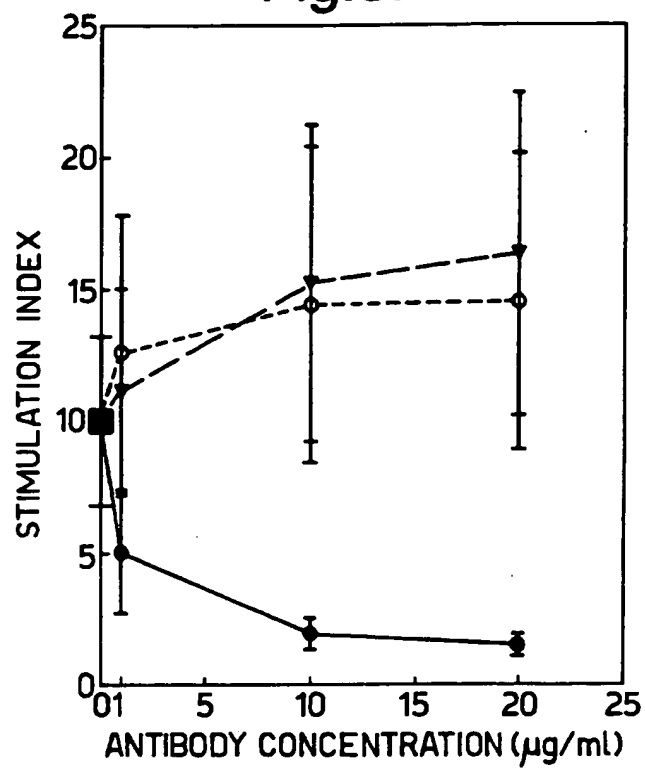
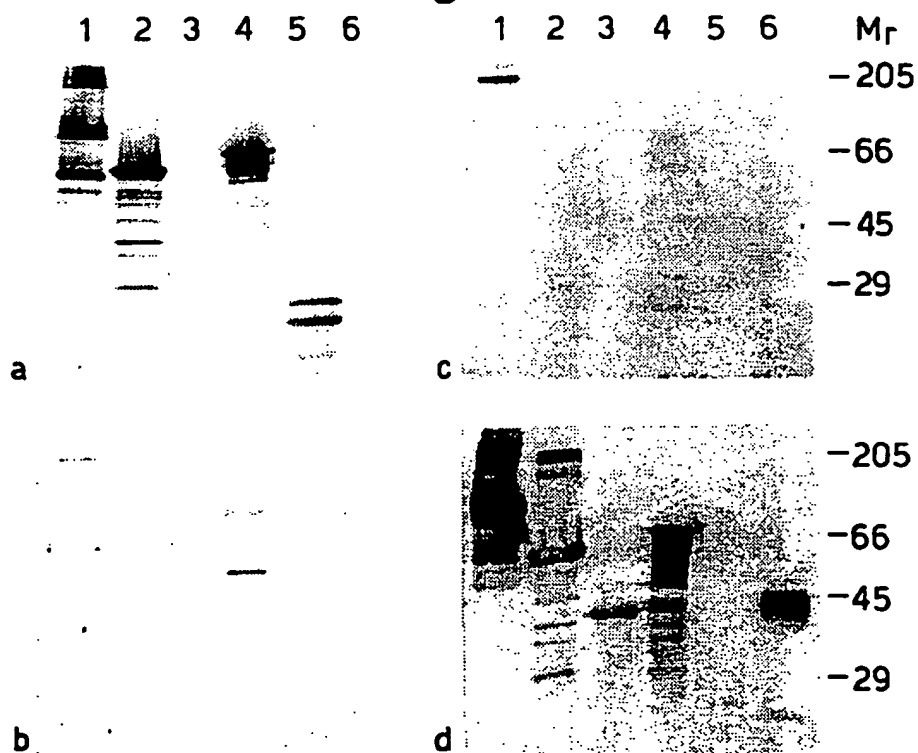


Fig.4.





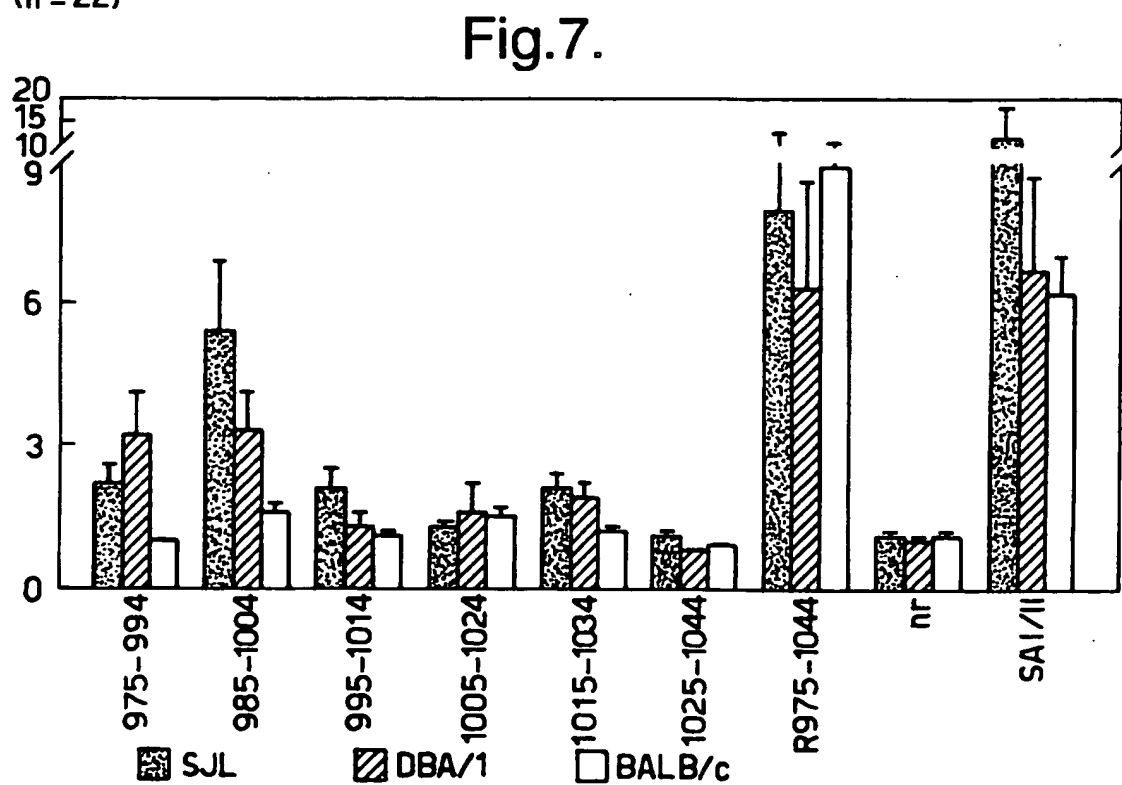
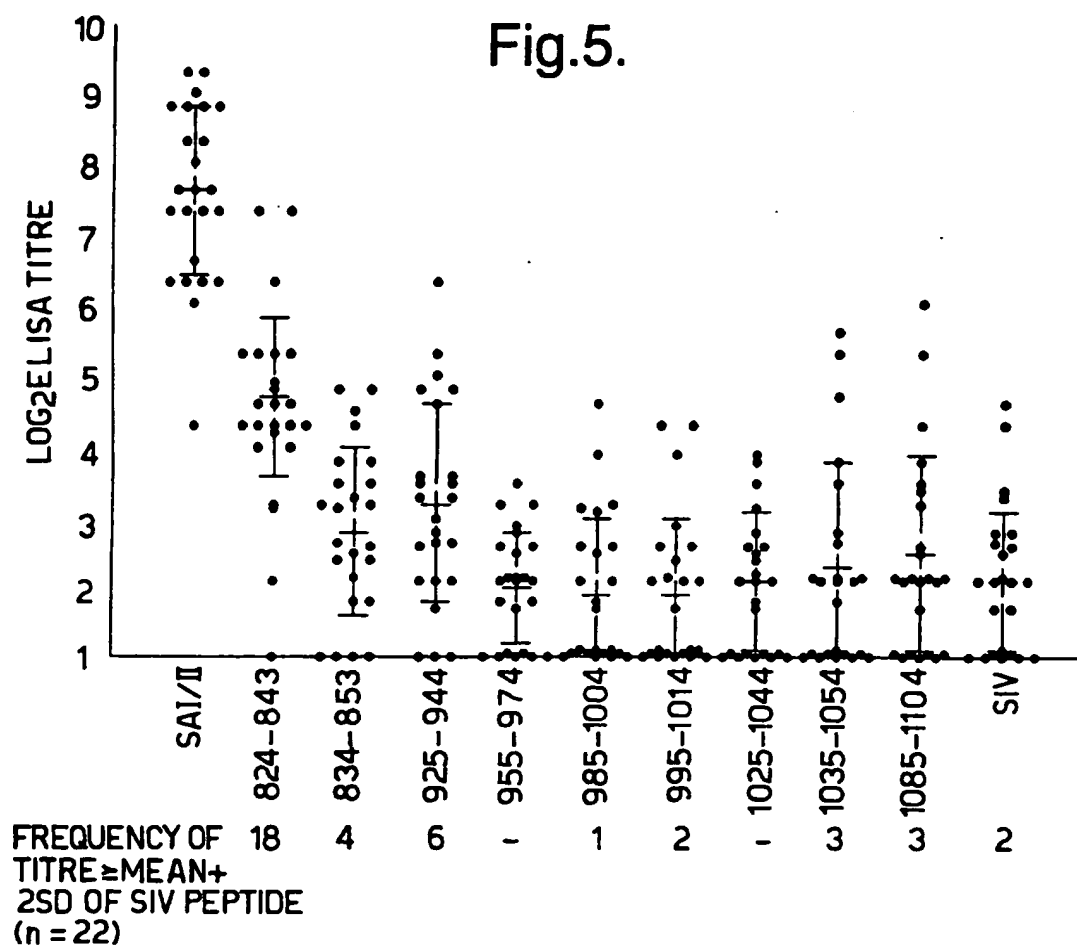


FIG. 6

RESULTS ARE MEAN  $\pm$  SD FROM 3 ASSAYS.  
CONCENTRATIONS OF INHIBITORS:

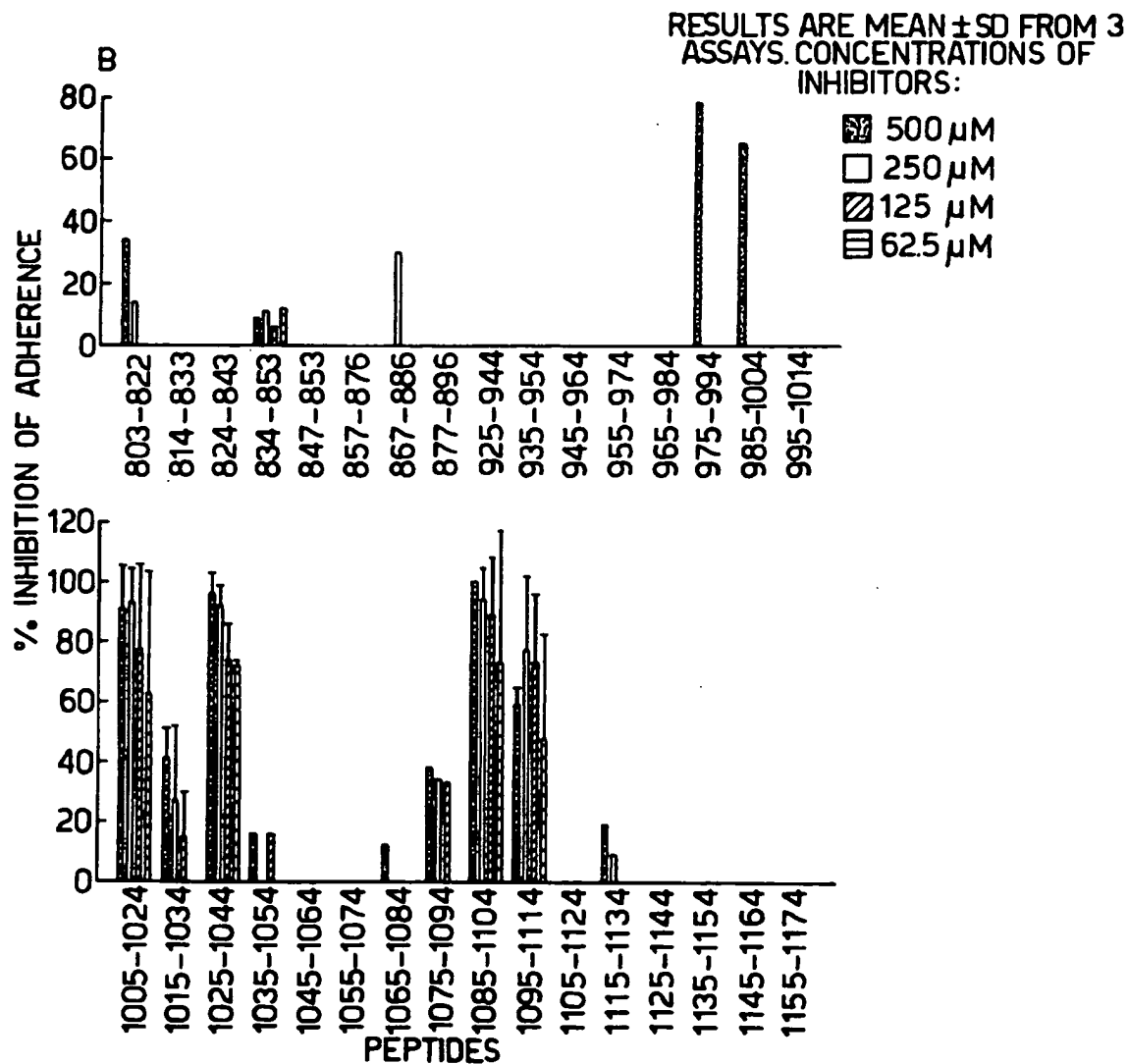
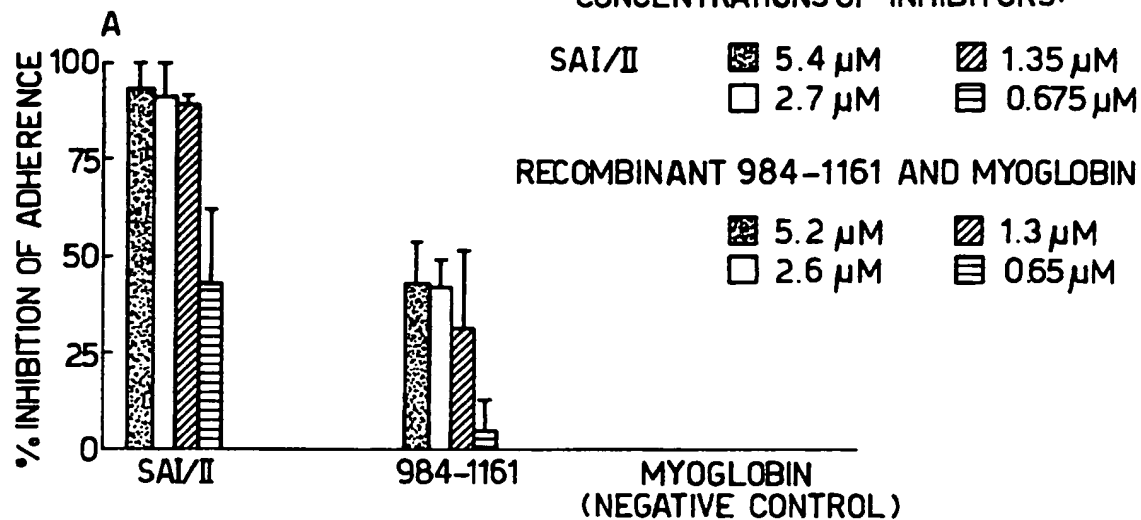


Fig.8.

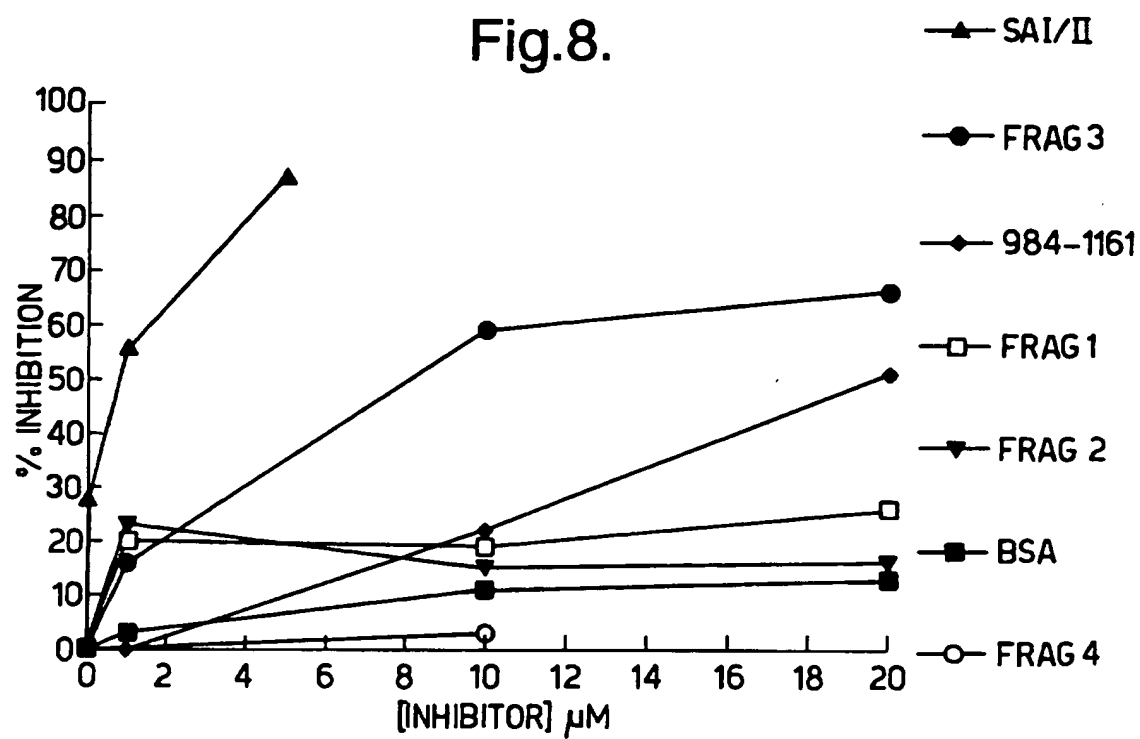


Fig.9.

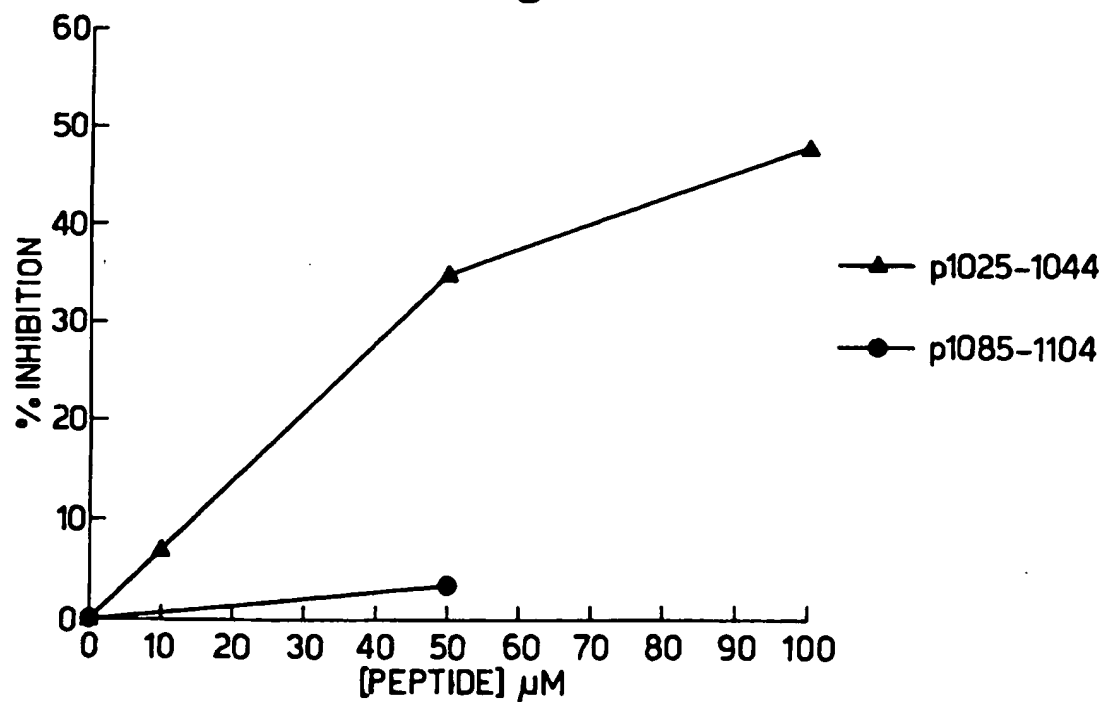


Fig.10.

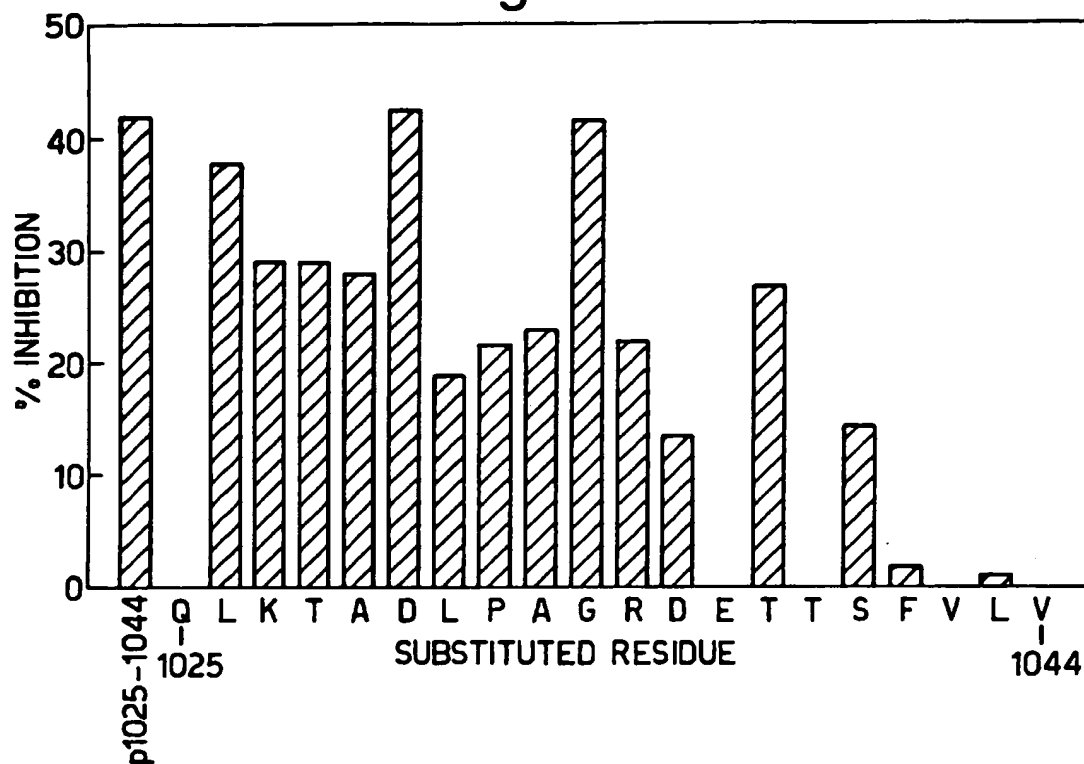
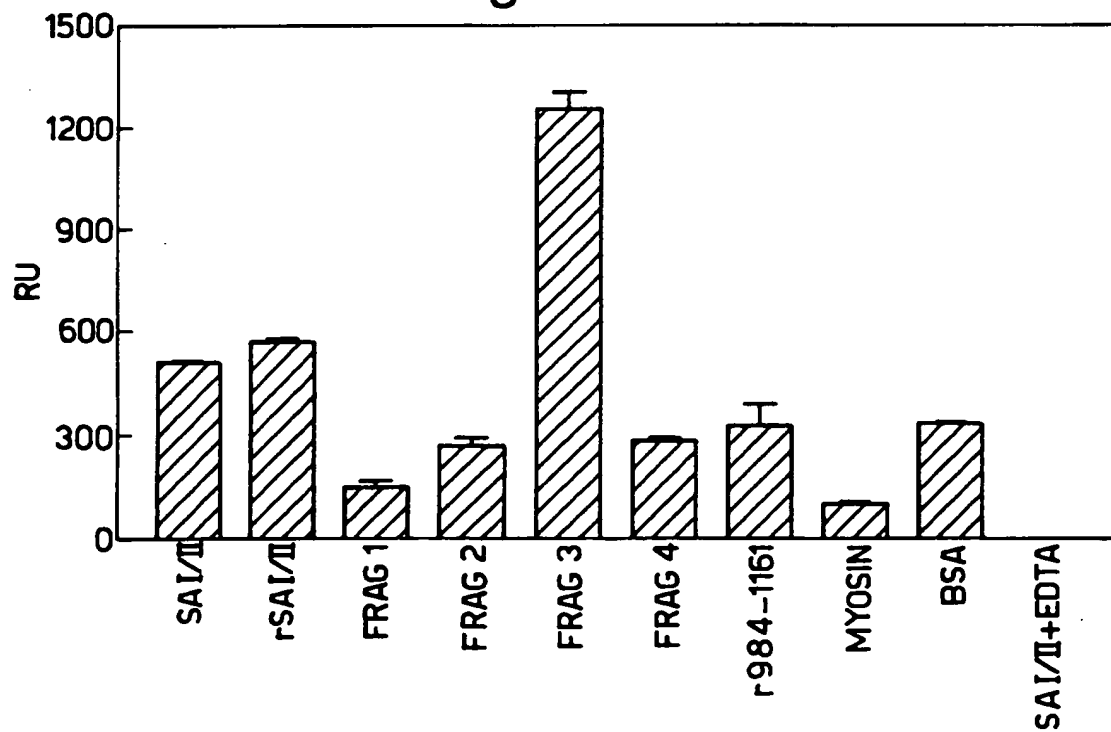


Fig.11.



## INTERNATIONAL SEARCH REPORT

Int ional Application No  
PCT/GB 96/00207

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12N15/31 C07K14/315 A61K39/09 //C12N15/62

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INFECTION AND IMMUNITY, (1994 JAN) 62 (1) 184-93, XP002003240 MOISSET, A. ET AL.: "Conservation of salivary glycoprotein-interacting and human immunoglobulin G-cross-reactive domains of antigen I / II in oral streptococci." see table 2	3,9,11
Y	---	1-9,11
Y	INFECTION AND IMMUNITY, (1993 NOV) 61 (11) 4590-8, XP002003241 MUNRO, G. ET AL.: "A protein fragment of streptococcal cell surface antigen I / II which prevents adhesion of Streptococcus mutans." see the whole document ---	1-9,11
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

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- "P" document published prior to the international filing date but later than the priority date claimed

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- "&" document member of the same patent family

Date of the actual completion of the international search

20 May 1996

Date of mailing of the international search report

17.06.96

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Authorized officer

Andres, S

# INTERNATIONAL SEARCH REPORT

In tional Application No  
PCT/GB 96/00207

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 280 576 (ST THOMAS HOSP MED SCHOOL ;JOHNSON & JOHNSON CONSUM PROD (US)) 31 August 1988 see the whole document ---	1-9,11
O,A	J.DENT.RES. 71 (IADR ABSTRACTS); 1992, 735, ABSTRACT 1753, XP002003347 MUNRO, G. ET AL.: "MAPPING OF ADHESION EPITOPES OF STREPTOCOCCAL ANTIGEN I - II." see abstract ---	1-9,11
O,A	J.DENT.RES. 72 (SPECIAL ISSUE; IADR ABSTRACTS), 1992, 517, ABSTRACT 17, XP002003242 KELLY, C. ET AL.: "GENETIC AND IMMUNOLOGICAL ANALYSIS OF CONSERVED EPITOPES OF ANTIGEN I - II IN ORAL STREPTOCOCCI." see abstract ---	1-6
A	GB,A,2 060 647 (RUSSELL M W;LEHNER T) 7 May 1981 cited in the application ---	
P,X	INFECTION AND IMMUNITY, (1995 SEP) 63 (9) 3649-58, XP002003243 KELLY, C. ET AL.: "T-cell, adhesion, and B-cell epitopes of the cell surface Streptococcus mutans protein antigen I/II." see the whole document -----	1-9,11

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/GB 96/00207

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0280576	31-08-88	AU-B- 1349588	26-09-88
		EP-A- 0346370	20-12-89
		WO-A- 8806594	07-09-88
		JP-T- 2502637	23-08-90
		ZA-A- 8801337	26-08-88
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GB-A-2060647	07-05-81	WO-A- 8101101	30-04-81
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